PERGAMON INTERNATIONAL LIBRARY
of Science, Technology, Engineering and Social Studies
The 1000-volume original paperback library in aid of education, industrial training and the enjoyment of leisure
Publisher: Robert Maxwell, M.C.

SOIL FUNGI AND SOIL FERTILITY

2ND EDITION

SOIL FUNGI AND SOIL FERTILITY

An introduction to soil mycology

S. D. GARRETT, Sc.D., F.R.S.
Emeritus Professor of Mycology, University of Cambridge, and Fellow of Magdalene College

2ND EDITION

PERGAMON PRESS
OXFORD · NEW YORK · TORONTO · SYDNEY · PARIS · FRANKFURT

U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
CANADA	Pergamon of Canada, Suite 104, 150 Consumers Road, Willowdale, Ontario M2J 1 P9, Canada
AUSTRALIA	Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W. 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg/Taunus, Hammerweg 6, Federal Republic of Germany

First edition 1963
Second edition 1981

British Library Cataloguing in Publication Data

Garrett, Stephen Denis
Soil fungi and soil fertility. – 2nd ed.
1. Soil fungi
I. Title
589′.2′09148 0R111 80–40802

ISBN 0–08–025507–8 Hardcover
ISBN 0–08–025506–X Flexicover

Printed in Great Britain by A. Wheaton & Co., Ltd., Exeter

TO MY WIFE

is dedicated in gratitude
this product of our life together

PREFACE

SINCE the first publication of this book in 1963, dramatic advances have been made in our knowledge and understanding of the way in which micro-organisms live and function, both under controlled conditions in the laboratory and in the natural environment of the soil. These advances have necessitated a replanning as well as a rewriting of this book, so that seven of the nine chapters are largely new. As before, this account of soil fungi is intended primarily as an introduction to soil microbiology for university undergraduates, and to show how the living ecosystem of the soil functions in the maintenance of soil fertility for crop production.

It is a pleasure to acknowledge the help I have had from others, by their critical reading and valuable comments on four chapters. Firstly, to my former research student Professor D. M. Griffin, of the Australian National University, for reading Chapter 2; in the subject that he has made so much his own, he has now been the teacher and I the taught. Secondly, to my colleague Dr. John Rishbeth, F.R.S., for reading Chapter 3 and also for all that I have learned from him in our common field of research on pathogenic root-infecting fungi. Thirdly, to my colleague Dr. H. J. Hudson, for reading Chapters 4 and 5; from his work and writings on fungal saprophytism I have learned much and now pass some of it on to others.

I am especially grateful to Mr. Brian Golding for three original drawings, now reproduced as Figs. 3, 5 and 8 and also for drawing the graphs shown in Fig. 11. I am indebted to authors, editors and publishers for permission to reproduce Figs. 1, 6, 9, 16, 17 and 18, sources of which are acknowledged individually in the text. Lastly I thank Mrs. Ruth Hockaday for the great skill and care with which she typed out my manuscript.

CHAPTER 1

INTRODUCTION

THIS book will be an introduction to the microbial ecology of the soil, using soil fungi as examples. Fungi have various advantages for ecological studies. They are the easiest micro-organisms to recognize and identify, which fits them for quantitative as well as qualitative investigations in soil. The fundamental study of soil fungi has been greatly encouraged by the economic importance of two groups of root-infecting fungi. The mycorrhizal fungi live on and in the roots of their host plants in a *symbiosis* that benefits both partners, because these fungi are more efficient in absorbing plant nutrients, especially phosphates, from infertile soils than are non-infected root systems. The pathogenic root-infecting fungi cause widespread and sometimes serious losses of yield in almost every crop all over the world. Nevertheless, even crop pathogens have their use as censors of bad agricultural practice. When I was working on the take-all disease of wheat in South Australia, in the early 1930s, it became obvious that intensive growing of wheat on the poorer, sandy soils was causing not only widespread losses from this disease, but also a decline in soil fertility and consequent wind erosion of these soils. Again in 1948, when working on Panama disease of bananas in Jamaica, I declared that the widespread abandonment of large tracts of land for banana growing, caused by this disease, had come in time to save much further loss of surface soil from the mountainous areas due to devastating water erosion. This opinion was not well received, nor had I expected it to be; blessings in disguise are not readily recognized by those who have suffered both financial loss and disruption of their traditional cropping practices.

In their mode of nutrition, fungi contrast with all green plants, which are

able to fix solar energy as carbohydrates through their possession of chlorophyll pigments. All such plants, from the largest forest trees down to micro-organisms like the blue-green photosynthetic bacteria (formerly styled the blue-green "algae"), are termed *autotrophic*, i.e. self-nourishing. Similarly, various groups of chemosynthetic bacteria, which derive their energy from the exothermic oxidation of sulphur and iron compounds, can also be called autotrophic. Fungi are called *heterotrophic*, because they derive their energy from the exothermic break-down of organic substrates produced by other organisms, either by the original autotrophs or by another organism along the food-chain. Fungi are not producers of energy; they are consumers. But they play a useful part in the maintenance of terrestrial life through their widespread activity on and in the soil. If soil organisms and micro-organisms did not play their essential role in the breaking-up and decomposition of dead vegetation on and within the soil, the soil surface would become littered with a deep layer of plant refuse, of little value as a source of nutrients for the root-systems of higher plants. Members of the soil fauna break up the plant remains into smaller fragments, which are thus more quickly degraded by the heterotrophic soil bacteria, which are surface-feeders. But it is the soil fungi that are typically the pioneer colonizers of dead plant tissues. They are fitted for this pioneering role by their physical organization into a network of *mycelium*, composed of branching, rigid tubes (*hyphae*), filled with protoplasm. Such hyphae are able to penetrate cellulose walls in plant tissue, even those strengthened by lignification, by a combination of enzyme action and growth pressure exerted by the hyphal apices, supported by a rigid mycelium. The unicellular bacteria do not have this mechanical advantage for penetration of intact though dead plant tissues, and so their cellulase enzymes can cause only surface erosion of cellulosic plant tissues; nevertheless, they can later enter dead plant tissues through punctures made by fungi, and through the more extensive breakage and comminution caused by the soil fauna.

So a corpus of dead plant tissue is gradually broken down by the commensal activities of the saprotrophic soil fauna and the heterotrophic soil fungi and bacteria. This word *commensal* means feeding "together at the table" and it implies no more than this. It must be carefully distinguished from the term *mutualistic symbiosis*, which means "living together" by two (occasionally more) partners with *mutual* advantage, e.g.

lichens, which are composite plants formed by association between fungi and green algae. The final result of this commensal break-down of dead plant tissue is the liberation of the essential mineral nutrients needed by higher plants in a soluble form, i.e. nitrates, phosphates, sulphates and salts of potassium, calcium and magnesium, which can be taken up from the soil by their root systems. So the essential mineral nutrients needed by plants, animals and micro-organisms, N, P, K, Ca, Mg, S and others in smaller quantities, are kept in circulation by the heterotrophic soil micro-organisms. We have to note, however, that successive generations of green plants cannot reclaim the carbohydrate energy-reserve that is locked up as cellulose, etc., within the skeleton of dead plant tissue. But these energy reserves indirectly benefit the green plant, because they provide the wages for the microbial work-force that reclaims in soluble form the mineral nutrients orginally locked up in the dead plant tissues.

So far we have emphasized the commensal nature of these break-down activities, partly because in the past, soil microbiologists have sometimes ignored the important part played by the soil fauna. Sometimes this has been done for research convenience, quite deliberately. The soil fauna of insects and other small arthropods, etc., add one more factor to an already complicated situation, and various kinds of insect larvae, for example, devour fungal mycelium and its fructifications on which identification of the species depends; it is possible to minimize this unwelcome activity by the simple expedient of air-drying soil or plant tissue before experimental use. But while recognizing the universal importance of commensal action in the soil, it is opportune to point out that fungi and bacteria typically occupy somewhat different *ecological niches* in the soil, at least as pioneer colonizers of dead plant and animal tissues, when their respective roles are more easily distinguished. In this respect, the supply of oxygen, i.e. its partial pressure, within the soil or inside a corpus of tissue, plays a differentiating role. In general, the fungi are strict *aerobes*; their activity is often limited by oxygen supply, though the yeast fungi constitute a well known exception. Some bacteria are as oxygen-demanding as the majority of fungi; others, known as *micro-aerophiles*, grow best in very low oxygen tensions, and there is a large and important group of *obligate anaerobes*, which will not grow at all in the presence of oxygen.

Such obligate anaerobes are the chief decomposers of mammalian corpses, the tissues of which quickly become anaerobic after death, when

the ventilating action of heart and lungs has ceased. The inoculum for decomposition is already there within the corpse, because the intestines harbour a large and active microflora of anaerobic bacteria. The high water content of the material in an animal's alimentary tract provides a favourable habitat for bacteria and the anaerobic condition of the intestinal contents limits the *active* microflora to anaerobic bacteria. Ruminant herbivores, such as cattle, have a highly evolved fermentation chamber in the anterior part of their alimentary tract; this is known as the *rumen*. The animal itself does not secrete cellulase enzymes but members of the bacterial population in the rumen do so and thus they can degrade the cellulose cell-walls in the well chewed fragments of herbage into soluble sugars, part of which is absorbed by the host animal after microbial transformation into fatty acids, together with digestion products of proteins, etc.; in this way, the herbivorous ruminant benefits by extracting a much higher proportion of the nutrients than it could do if unaided by the bacterial microflora of its digestive tract. This association is clearly a symbiosis; the benefits to the host animal of cellulose digestion are obvious, and the bacterial population is provided both with a food substrate and optimum conditions for its decomposition. Evolution of the rumen seems to be a remarkable adaptation to a herbivorous diet; the wild rabbit has no rumen but it regularly devours its own dung balls, thus submitting them to a further extraction of residual nutrients.

These examples have been chosen to show that sometimes a single master factor, in this case oxygen supply, may broadly differentiate between several groups of micro-organisms, all of which may be at least potential competitors for a corpus of dead plant or animal tissue providing a *substrate*, i.e. food material for micro-organisms. Usually, however, many factors in both substrate and environment act together in selecting the micro-organisms best fitted for colonization of a particular ecological niche. Substrate factors comprise physical organization, chemical composition and relative availability of nutrients. Factors of the soil environment include temperature, degree of aeration, availability of water, chemical composition and availability of mineral nutrients, and hydrogen ion concentration (pH value). But before we can understand the complex organization of microbial activities within the soil, it is essential to understand, at least in broad outline, the physical framework and chemical

composition of the soil. This phrase "to understand" is here used in a relative (or optimistic) sense and it will soon appear that much remains to be found out about the behaviour of soil by physicists, chemists and microbiologists.

CHAPTER 2

SOIL AS A HABITAT

The shortest definition of "soil" that I have seen was produced by G. V. Jacks (1954) in his book *Soil*: "Soil is what plants grow in". To explain clearly the physical and chemical framework of soil as a habitat for organisms, it is unfortunately necessary to subdivide aspects of the subject that are not naturally divisible; there are no sharp demarcations in the natural world but they are still necessary for the exposition and administration of science.

PHYSICAL CHARACTERISTICS OF SOIL

Soil texture

This term expresses the distribution of the ultimate particles of a soil within a range of conventionally determined sizes, as shown in Table 1.

TABLE 1. GRADING OF SOIL MINERAL PARTICLES

Grade	Particle diameter (mm)
Gravel	>2
Coarse sand	2–0.2
Fine sand	0.2–0.02
Silt	0.02–0.002
Clay	< 0.002

The size distribution of the particles is determined by a method known as *mechanical analysis* of soil. Particles are separated by sieving for grades

down to and including coarse sand, the weight of which in the soil sample is determined after drying. Separation of the finer grades depends on the fact that the finer a mineral particle, the more slowly does it fall through water. The time taken by spherical particles to sediment through a standard vertical column of water at a standard temperature can be calculated from Stokes's equation. Although the mineral particles are far from spherical and their mean density has to be conventionally assumed as 2.6, yet these assumptions do not invalidate the method for comparison between different soils. The method of analysis begins with crushing the soil, so that as much as possible will pass through a sieve of 2 mm mesh. The organic matter is first removed by boiling the soil in a solution of hydrogen peroxide; the organic matter content is determined on another sample of soil. Calcium carbonate is then removed by leaching with dilute hydrochloric acid; this has to be done because calcium salts tend to flocculate the mineral particles, i.e. cause them to cohere into aggregates. Any soil aggregates still remaining are finally dispersed by shaking the soil sample in a dilute alkali solution, after which the fraction of coarse sand is separated off by wet sieving and the remainder is suspended in a vertical column of water. At suitable intervals, the density of the soil suspension at a fixed depth is determined, most quickly and easily with a hydrometer, the use of which for this purpose was introduced by G. J. Bouyoucos in 1927. From the results, the distribution of the ultimate mineral particles between the various grades can be calculated.

The results of such mechanical analyses of soils are needed particularly by pedologists in their work on the evolution and classification of soils. But the experienced soil surveyor can often make quite a good guess at the mechanical composition by merely examining and handling the moist soil, just as an experienced statistician can run his eye over a table of data and assign a probable degree of significance for differences between means. Those who enjoy country walks learn more about soil texture from their feet than they may consciously realize. Thus it is possible to run across a field of bare sandy soil without much effort and without getting the legs dirty; if a similar traverse is made across a field of wet clay, especially if recently ploughed, the first such experience will not soon be forgotten. Such a sandy soil is often referred to as "light-textured" or "light", whereas a clay soil is similarly called "heavy-textured". These fairly ancient terms have nothing to do with what is called "bulk density" of a soil; they refer to

the number of horses required to pull a plough through the moist soil, which would have been only one for most sandy soils and up to four for a clay soil. But the draught of a plough is not determined by soil texture alone in any particular soil at any particular time, because it is affected by the state of aggregation of the soil particles to give the *soil structure* (see next section). The individual farmer has to decide when a heavy clay soil is at the optimum moisture content for ploughing, i.e. when the power required to draw the plough is least; through the correct use and timing of his cultivations he can create and preserve an optimum soil structure, with resulting economy in cost of power. Lastly, we should note that soils intermediate in texture between sands and clays are usually described as *loams*. A loam soil usually contains not more than *ca.* 30 % clay along with 40–50 % sand, but sub-divisions are often made, not always accurately I suspect, into light loams, medium loams and clay-loams.

Soil structure

Most soils have a definite and visible structure; although various types of soil structure have been described, especially for natural soils under wild vegetation, that which is deemed best for agriculture is a granular or *crumb structure*. The soil crumbs, like the ultimate mineral particles, vary over quite a wide size-range (optimum 1–5 mm diam.) in different types of soil, and also within one soil at different times, according to management by the farmer. In each crumb, the coarse particles are usually surrounded by fine particles, which cement the crumb together through the surface forces associated with fine particles of clay and humus. A cultivated soil in good *tilth*, as the farmer would say, is made up of an assemblage of variously sized soil crumbs, separated by a network of intervening *soil pores*. In a soil at medium moisture content, the soil pore space is occupied by air and water (more strictly speaking, by the soil solution) in roughly equal proportions. Micro-organisms live both within the pore spaces and also in the interior of the crumbs, which provide a microbial environment somewhat different from that in the pore spaces; the centre of a water-saturated crumb is likely to be anaerobic if the crumb radius exceeds *ca.*3 mm. The total population of bacteria in a soil at any time is probably limited not only by available substrates providing nutrients, but also by the total internal surface area, both within and outside the soil crumbs, per unit

volume of soil. This is because bacteria, much more than fungi, are restricted to growth in films of liquid covering internal soil surfaces. As noted above, the structure of a soil can be much influenced, for better or for worse, by a farmer's crop-husbandry operations. Grassland is one of the best makers and preservers of good crumb structure and so a good arable rotation generally includes a temporary pasture, or ley, usually made up of one or more grasses along with a legume, for one or more years. The mechanical effects of the growth of a mat of fine grass roots may contribute to this effect, in addition to the fact that roots produce an external coating of mucilage. Young grass roots, like young roots in general, exude a solution that serves as a more or less complete nutrient medium for growth of micro-organisms. This increased population of micro-organisms around the young, active roots is known as the *rhizosphere*. Many species of soil bacteria produce a sheath of polysaccharide substances around their cells and such gummy excretions can help the building up of soil crumbs, as can the binding effect of fungal mycelium and the organic residues left when the mycelium dies and autolyses. Such possible effects of soil micro-organisms on crumb structure have been critically reviewed by Griffiths (1965).

Soil water content

It is quite easy to determine the moisture content of a sample of soil, by weighing it before, and again after, it has been air-dried or oven-dried. For any collection of soil to be used for experimental work in soil microbiology, it is necessary to calculate moisture content on an air-dry basis, because soil is denatured by oven-drying and is then quite useless for microbiological studies. Anyone who looks after potted plants in a glasshouse soon learns from experience when plants need watering; by the appearance and feel of the soil one can say whether it is too dry, about right or too wet, and for this purpose no precise determination of soil moisture content is needed. But a statement that an unknown soil contains a certain percentage of water is usually meaningless, because the weight of water that can be taken up and held by 100 g air-dry soil at saturation usually varies over the range 25–80 g. From about the year 1920 onwards, therefore, microbiologists determined the saturation capacity of any stock of air-dried soil to be used in laboratory experiments, employing the *perforated box method* described by Keen and Raczkowski (1921), or some modification of it. Rectangular

boxes with a perforated bottom, lined with a sheet of thin filter-paper, were weighed before and after filling with air-dried soil, and then stood in a shallow layer of water until the soil was saturated; then the boxes were re-weighed. From the figures thus obtained, the weight of water held by 100 g air-dry soil could be calculated. From this figure, the volume of water required to bring *ca.*200 ml air-dried soil to a medium moisture content, of 50–60 % saturation, could be calculated. Cylindrical glass jars of *ca.*300 ml capacity were used as soil containers; if the required volume of water was added not too quickly to the surface of the air-dried soil, it would percolate downwards, and soil moisture would become evenly distributed within 24–48 h, as shown by determining actual moisture content of consecutive slices of soil. Those employing this method of preparing moist soil in small containers usually experienced no difficulty in getting uniform packing of soil, previously passed through a 2 mm sieve, or uniform distribution of water down to a moisture content of 50 % saturation. Some types of soil might be difficult to handle and so were unsuitable for this kind of laboratory work. But at soil moisture contents below *ca.*50 % saturation, distribution of soil moisture was less good and failed to become even through the soil. So most workers soon realized that this method was unsuitable for study of the effect of soil moisture content upon microbial activity and other methods had to be found.

The procedure outlined above made it possible to bring a soil to a medium or high moisture content, and to maintain it there by periodical addition of de-ionized water to return soil containers to their original weights, i.e. to replace water lost by evaporation from the soil surface. So the effect of experimental treatments upon microbial activity in one particular soil could be compared, and experimental results were usually repeatable in consecutive experiments with the same stock of air-dried soil. But eventually it was realized that different types of soil held at the same moisture content, as expressed by percentage saturation, were not at equivalent moisture contents with respect to availability of water to plant roots and micro-organisms. To withdraw water from a soil at a moisture content of less than saturation, roots and micro-organisms have to exert a suction, and the magnitude of the required suction increases as the soil gets drier. The tenacity with which a soil holds water against the demand of roots and micro-organisms varies widely according to the type of soil; the finer the soil particles, the more strongly is water held by surface forces.

Thus a quartz sand holds only *ca.*20 g water/100 g sand at saturation, but only a low suction, of *ca.* −1 atm, is required to withdraw nearly all the water. A clay soil, on the other hand, may hold up to 80 g water/100 g soil at saturation, but a suction of −1000 atm will be needed to withdraw nearly all of the water. At a moisture content of 30% saturation, a suction of *ca.* −0.5 atm will be needed to withdraw water from the sand, whereas one of *ca.* −10 atm will be required to withdraw it from the clay soil. So it becomes clear that the expression of soil moisture content as percentage saturation gives little information about the availability of water to roots and micro-organisms and so it does not afford a valid basis for comparison between different soils over a range of moisture contents.

This then new concept about the state of water in soil and its measurement was embodied by R. K. Schofield (1935), a soil physicist at the Rothamsted Experimental Station, in a paper entitled "The pF of the water in soil". Schofield expressed the suction needed to withdraw water from a particular soil at a given moisture content (g water/100 g soil dried at 105°C) on a logarithmic scale, on which pF 0 = 1 cm water suction, pF 1 = 10 cm and pF 7 = 10^7 cm water suction. Expression of pF on a $\log_{10}$ scale is thus analogous to the expression of hydrogen ion concentration on the pH scale.

The new ideas concerning soil water content developed by Schofield and others took a long time to penetrate current laboratory practice in soil microbiology; soil physicists may well have said that it took a *very* long time. Adoption of a new technique, however, involves more than the assimilation and adoption of a new idea; it also means getting used to a possibly more difficult and laborious method. At that time, measurement of soil pF involved the use of a water tensiometer. Much was done by D. M. Griffin (1963) in a paper entitled "Soil moisture and the ecology of soil fungi" to persuade soil mycologists and microbiologists to use the new methods; since that time their assimilation has been quite rapid (Cook and Papendick, 1970).

The form of the relationship between the actual moisture content of a soil (g water/100 g oven-dry soil) and pF value is shown in Fig. 1, reproduced from Griffin's (1963) paper.

The curves shown for a sand, a loam and a clay soil in Fig. 1 are known as *moisture characteristic* curves; those for sand and clay represent the extremes of relationship between moisture content and soil pF, with the

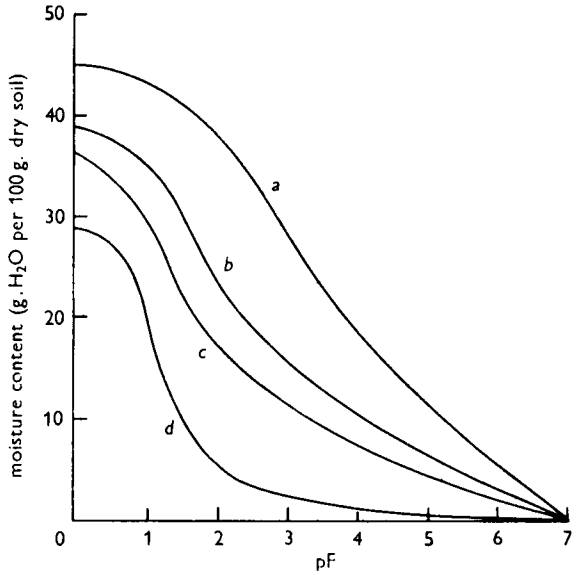

FIG. 1. Representative moisture characterisitics: *a*, drying boundary curve of a clay; *b*, drying boundary curve of a loam; *c*, wetting boundary curve of a loam; *d*, drying boundary curve of a sand (reproduced from D. M. Griffin (1963), *Biological Reviews*, Cambridge).

loam as an intermediate. As Griffin has explained, the moisture characteristic curves for collections of soil from one locality may vary with structure (a variable characteristic) as well as with texture, and also with the past history of wetting and drying of the soil. The latter point is illustrated by the two central curves in Fig. 1; *b* represents the drying boundary-curve for the loam soil drying out from saturation, and *c* the wetting boundary-curve for the same soil taking up water from the oven-dry condition. The difference between these two boundary curves, with possible intermediates, is due to a phenomenon termed *hysteresis*, which need not be explained here; its existence should be remembered, however, together with the fact that the drying boundary-curve is easier to determine, because of the greater speed of equilibration between soil and water.

To complete this explanation of the behaviour of water in soil, and to bring it up to date, just a few further points have to be mentioned. The state of water in soil is now generally expressed not on the pF scale but on that of

a related scale, known as the *water potential*; in a soil less than saturated with water, the water potential has a negative value. Water, like heat, flows from regions of high to regions of lower energy. Pure, free water at atmospheric pressure is assigned a value of zero potential energy. Because work has to be done by applying suction, to withdraw water from soil at a moisture content less than saturation, the potential of the soil water has a negative value. Just so, pure free water with zero potential will flow into unsaturated soil with a negative potential. At one time, before the introduction of S.I. units (*Système International d'Unités*) water potential would have been expressed in terms of a negative value of atmospheric pressure, e.g. −1, −2, etc. atm. I have used this older terminology earlier in this discussion, because to many microbiologists it is still more familiar than the new units. The new S.I. unit is the *pascal* and 1 MPa (megapascal) = 9.87 atm. An alternative unit, the *bar* (1 bar = 0.987 atm.) has been employed until recently, but under S.I. recommendation should now be restricted to meteorology; its use in soil science is thus obsolescent.

Lastly, the forces that hold water in soil can be briefly categorized. Over the range of soil moisture content in which microbiologists are likely to be interested, i.e. from saturation down to air-dry in an arid climate, these are of two types. Firstly, there is an osmotic effect due to solutes in the soil solution associated with colloidal particles of clay and humus; this imparts a negative *osmotic potential* to the moist soil. In saline soils characteristic of irrigated areas in arid climates, this negative osmotic potential may be considerable, but it is often negligible in temperate climates except where an excess of chemical fertilizers has been applied. More usually, much the larger component of total water potential is *matric potential*. The negative value of matric potential is due to the forces that act at air-water and solid-water interfaces. These forces are sometimes called *capillary forces*, because in part they are similar to that causing water to rise within a vertical capillary tube. In part, however, matric potential is due to the forces that bind water to the mineral particles, aggregates and organic material that together make up the soil.

Methods for measuring and controlling water potential in soil have been described by D. M. Griffin (1972) in his book *Ecology of Soil Fungi*. Use of an instrument like the thermocouple psychrometer will eventually become as widespread in both field and laboratory studies as is now the use of a pH-meter. Griffin's book can be strongly recommended as an introduction to

soil physics for microbiologists. All physical factors are treated in detail and in depth, but Griffin's accounts are so arranged that those unable or unwilling to follow mathematical and physical theory can still read this book with much profit. These remarks can be kept in mind for the section now to follow.

The soil atmosphere

In a soil at medium moisture content, about half of the soil pore space will be filled with liquid and the remainder with the soil atmosphere. When soil is in a state of activity from the growth of roots and decomposition of organic substrates by heterotrophic micro-organisms, the average partial pressure of carbon dioxide in the soil atmosphere is likely to be somewhat higher than that of the air above ground, and the partial pressure of oxygen may be slightly lower. But around respiring roots and their rhizospheres of micro-organisms, these differences may be much accentuated. Griffin (1972) has pointed to an important distinction between soil fungi and bacteria in respect of soil location. By their organization as a rigid mycelium, fungi are enabled to grow across and to occupy air-filled pore spaces; bacteria are obliged by their unicellular organization to grow sessile on the internal surfaces of the soil, around and within the soil crumbs. For active growth, bacteria require a film of liquid covering soil surfaces, which must be deep enough to accommodate their cells, i.e. a minimum of 2 μm. Griffin has concluded that, in most natural soils, bacterial growth and activity is likely to be restricted at water potentials less than -0.5 to -5 bar, whereas many kinds of soil fungi can tolerate soil water potentials lower than this. Griffin (1968) had earlier shown, by microscopical observations of fungi growing in a translucent particulate matrix of glass micro-beads, that fungi such as *Cochliobolus sativus* and *Curvularia* sp. sporulated only in air-filled spaces large enough to accommodate their rather large spores. But another species, *Fusarium culmorum*, produced its masses of slime spores within the liquid films though adjacent to the liquid-air interface.

The question of the precise location of micro-organisms within the soil has aroused much interest in recent years, particularly with regard to composition of the soil atmosphere within micro-habitats such as the surface of growing roots. The whole situation has been much clarified in a

paper by D. J. Greenwood (1970) entitled "Distribution of carbon dioxide in the aqueous phase of aerobic soils". Firstly, Greenwood has pointed out that, in air channels directly connected with the atmosphere above the soil, equilibrium with the outside atmosphere will be quickly restored by gaseous diffusion, so it is most unlikely that growth of either roots or micro-organisms will be reduced either by excess of carbon dioxide or by lack of oxygen in such soil air-channels. But within the liquid films lining soil pore-spaces at medium soil moisture contents, and surrounding growing roots, molecular equivalents of oxygen absorbed will be balanced by molecular equivalents of carbon dioxide released; this balance will be upset only within oxygen-free micro-sites, in which anaerobic respiration will occur. But speed of diffusion of these two gases through water films will depend upon (1) diffusion coefficient of the gas (2) its solubility in water. The ratio of the diffusion coefficients, carbon dioxide/oxygen, is 0.74 at 10°C; the ratio of the solubilities in water at 10° is 31.4, and neither ratio is appreciably affected by temperature. So the ratio between rates of diffusion through water films by these two gases is given by the product of the above two ratios, i.e. $0.74 \times 31.4 = 23$. So carbon dioxide should move away from a respiring root in a water film 23 times more quickly than oxygen will move in, to restore the equilibrium in partial pressure. Greenwood has calculated that when the partial pressure of oxygen in a water film has been reduced to zero, that of carbon dioxide is unlikely to exceed 0.01 atm, i.e. 1% by volume. This partial pressure of carbon dioxide is too low to affect adversely the growth of either plant roots or soil micro-organisms. Greenwood confirmed these conclusions by measuring partial pressures of oxygen and carbon dioxide in a variety of model systems. So it appears that growth of both plant roots and soil micro-organisms is likely to become limited by a lack of oxygen *before* it becomes checked by an excess of carbon dioxide.

This conclusion has seriously upset earlier arguments, by soil mycologists in particular, that carbon dioxide, especially at lower levels in the soil and in micro-sites of rapid respiration, may increase to a partial pressure at which it becomes fungistatic. As it is possible to discuss one's own mistakes with more assurance and less charity than would be appropriate for those made by other workers, I will illustrate the point at issue with an early hypothesis of my own, which Greenwood's (1970) paper made untenable. From 1933 onwards, I had been measuring the speed at which the take-all

fungus, *Gaeumannomyces graminis* var. *tritici* (formerly known as *Ophiobolus graminis*) grows along the roots of wheat seedlings maintained in small containers of soil; the fungus grows over the roots as a mantle of dark-coloured mycelium, sending infection-hyphae into the root cortex as it grows along the outside (see Fig. 16, Ch. 9). From all my data taken together, I concluded (Garrett, 1936) that the most rapid growth of *G. graminis* along wheat roots was favoured by (1) good soil aeration (2) a slightly alkaline condition of the soil (pH > 7.0). These conditions agreed well with those already known to favour rapid development of the take-all disease of wheat and barley crops in the field. To explain these results, I proposed a unifying hypothesis, viz. that growth rate of *G. graminis* was limited by the partial pressure of undissociated carbon dioxide at the root surface; this constitutes a micro-site where oxygen is being absorbed and carbon dioxide released through the respiration of both the plant root and its rhizosphere of micro-organisms, including *G. graminis*, subsisting on root exudates. The role of soil pH in this relationship was ascribed to the complete change in the balance between undissociated carbon dioxide and the bicarbonate ion (HCO_3^-) over the range between pH 5.5 (90 % CO_2) and pH 8.0 (5 % CO_2); my hypothesis assumed that undissociated carbon dioxide rather than the bicarbonate ion was fungistatic towards *G. graminis*. In further experiments to test this hypothesis (Garrett, 1937), growth rate of *G. graminis* along roots of wheat seedlings was tested under two different sets of conditions (1) in glass tumblers of soil held at a moisture content of 70 % saturation (as in earlier experiments), which limits the degree of soil aeration (2) in glass tubes filled with soil at a lower moisture content (50 % saturation), to facilitate the continuous passage of air forced through the soil by a pump. After 14 days, mean distances grown by *G. graminis* along wheat seedling roots through various types of soil, under conditions of both poor and good aeration, were measured (Fig. 2).

The results shown in Fig. 2 appeared to confirm my general conclusion that soil aeration was the master factor controlling speed of growth by *G. graminis* along roots, especially as soil pH scarcely affected fungal growth rate in the set of forcibly aerated soils. My hypothesis remained unchallenged for more than 30 years, until I learned of the work by D. J. Greenwood, several years before the publication of his (1970) paper. So I suggested to my future research associate, Dr. J. F. P. Ferraz, that he should re-investigate the whole problem in the light of Greenwood's

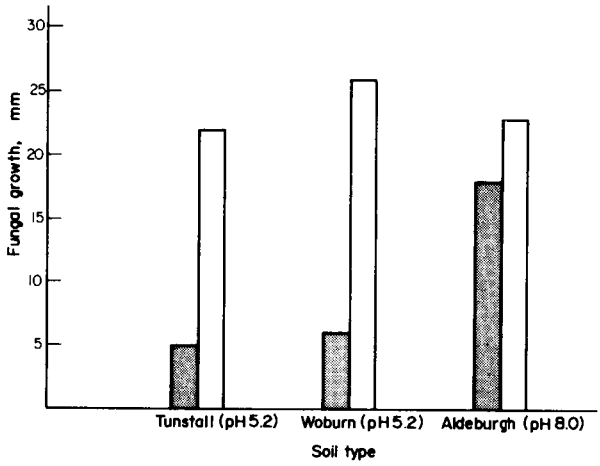

FIG. 2. Distances (mm) grown by *Gaeumannomyces graminis* var. *tritici* along wheat-seedling roots in three types of soil, under conditions of poor aeration (shaded columns) and good aeration (open columns).

conclusions. Ferraz (1973) conducted his experiments in two types of soil, each collected from the field and then adjusted to a range of pH values from 5 to 8. As a carbon-dioxide absorbant he employed a liquid surface of a saturated aqueous solution of sodium hydroxide, which was placed in diffusion contact with the soil atmosphere; by this technique, carbon dioxide content of the soil atmosphere was reduced to $< 0.2\%$, a biologically negligible level, as shown by periodical gas analyses of the soil atmosphere. In none of the experiments with these two soils, treated only for adjustment of soil pH and not receiving any addition of fresh organic material, did growth of *G. graminis* show any significant response to removal of carbon dioxide from the soil atmosphere by the absorbant. So for this particular test case we fully confirmed Greenwood's (1970) conclusion that growth of a soil micro-organism within a water film is likely to become retarded by a lack of oxygen before it is affected by an excess of carbon dioxide.

This conclusion does not necessarily hold true for soil that has recently received a heavy addition of quickly decomposable organic material. Thus Ferraz (1973) studied the effect of adding 1% (*w*/*w*) of finely divided grassmeal to the soil, an addition far larger than would ever be made in field practice. After 3 days, percentage volume of carbon dioxide in the soil

atmosphere rose to 5.4 in soil at pH 5.0, and to 3.0 in the same soil at pH 7.9. Under these circumstances, growth rate of *G. graminis* along roots was substantially increased by presence of the carbon-dioxide absorbant, so that an excess of carbon dioxide must have been reducing fungal growth in absence of the absorbant. Addition of such fresh organic material to soil causes an explosive outburst of microbial respiration, with the creation of temporarily anaerobic micro-sites. Under such conditions, volatile by-products of microbial metabolism other than carbon dioxide may also be produced and exercise fungistatic and bacteriostatic effects. The work of A. M. Smith (1976) has focused attention on the role of ethylene as a possibly widespread regulator of microbial growth in anaerobic micro-sites.

CHEMICAL CHARACTERISTICS OF SOIL

In the coarser fractions of soil, down to and including silt, the commonest mineral particles are those of quartz or silica (SiO_2), largely because they are chemically inert and thus the mineral that is most resistant to decomposition by weathering. Also common are the felspars, which are aluminosilicates of potash, soda and/or calcium; the micas, also common minerals, are another kind of aluminosilicate of potash, magnesia and/or iron. The clay minerals are not found in unweathered rocks and are composed chiefly of minerals produced by the weathering process; they are described as crystalline, hydrous aluminosilicates. The montmorillonites are characteristic of an early stage of weathering, and their particles are the most active, both chemically and physically. The micas are typical of an intermediate and the kaolinites of an advanced stage of weathering; in general, chemical activity decreases with the degree of weathering. Irrespective of mineral composition, it is the colloidal particles of clay and humus that are chemically the most active in soil; on account of their very high surface/volume ratio, their aggregate surface area in most soils usually much exceeds that of all the other soil fractions put together.

Base exchange of cations by soil colloids

The phenomenon of "base exchange" in soils was first discovered by J. T. Way in 1850 when investigating the chemical fertilizers introduced by

Lawes and Gilbert into their field experiments at Rothamsted around 1843. Way found that if soil was percolated with a solution of sulphate of ammonia ($(NH_4)_2SO_4$), the drainage liquid consisted largely of calcium sulphate ($CaSO_4$) and contained no ammonium sulphate. Evidently the calcium ions, Ca^{++}, in the soil had been "exchanged" for the added ammonium ions, NH_4^+, which were retained by the soil. Way further showed that potassium ions, K^+, were similarly retained by soil but that nitrate ions, NO_3^-, were not.

It was later shown that this property of base exchange, or *cation exchange* as it is now called, resides in the colloidal particles of clay and humus, which fundamentally are negatively charged by acidic anions of complex silicic and humic acids. These negative charges are balanced by the *diffuse double layer*, as it is called, of positively charged cations of Ca^{++}, Mg^{++}, K^+, NH_4^+ and H^+, etc. These cations of low valency are not tightly bound to the colloidal particle but occur as a diffuse layer in the soil solution around the particle, of a width estimated as varying from 2 to 20 nm. If soil is saturated with an excess of ammonium sulphate solution, for example, most of the cations will be displaced by NH_4^+, according to the chemical law of mass action. The domestic water softener originally invented under the name of "Permutite" made use of this process of cation exchange. "Hardness" in water supplies occurs when the water is collected from limestone areas and is due chiefly to the occurrence of bicarbonates and sulphates of calcium and magnesium in the water. By the Permutite process, the water supply is passed through a cylinder filled with permutite, a sodium aluminosilicate.This material takes up calcium and magnesium ions from the water, and replaces them with sodium ions; the emerging water contains sodium bicarbonate and sulphate and has lost the objectionable characteristics of "hardness". The permutite is regenerated at intervals by cycling through it a strong solution of sodium chloride.

In most cultivated soils of temperate regions, the predominant exchangeable cation is that of calcium. In many cultivated soils over the British Isles, percentages of exchangeable cations are of the following order: calcium, 80–90; magnesium, 5–10; potassium, 2–3; sodium, 0–5. The proportion of hydrogen ions varies widely, according to the pH value of the soil. The predominance of calcium is deliberately maintained by the farmer through periodical addition of lime, mainly to prevent the development of soil acidity. But the use of lime ($Ca(OH)_2$) for this purpose

has a great additional advantage, because calcium causes the colloidal particles in soil to aggregate, or to *flocculate*. This promotes crumb formation, and hence a better soil structure, which makes for ease of cultivation and so saves energy. In many cultivated soils of arid regions, which have to be irrigated for growth of crops, the predominant ion is that of sodium; this has an effect on soil structure opposite to that of calcium, because sodium tends to deflocculate the soil colloids. Such soils are very difficult to work, becoming sticky when wet, and drying down into rock-like clods. But they can be reclaimed by treatment with gypsum ($CaSO_4$), whereby the sodium is exchanged for calcium.

As noted above the most active, physico-chemically, of the clay minerals are the montmorillonites. They are three-layered hydrous aluminosilicates, which swell on wetting; when thus wetted, the plate-like layers move apart sufficiently to expose at least part of their internal surfaces, thus increasing their activity in cation exchange. The specific surface of montmorillonites has been estimated as *ca.*800 m^2/g; this can be compared with a value of *ca.*50 m^2/g for the kaolinites, which have the lowest surface activity amongst the clay minerals. The surface activity of montmorillonites is thus about 16x that of kaolinites; Stotzky and Rem (1966) have estimated the ratio of their respective cation exchange capacities to be of the order 17–20:1.

Owing to this large array of exchangeable cations held by colloidal particles in montmorillonite clays, such soils have an unusually high *buffering capacity* for stabilization of soil pH around or slightly above neutrality. So when an excess of hydrogen ions is produced by microbial metabolism, this potential excess is quickly taken up from the soil solution through cation exchange around the surfaces of the clay particles. Most soil bacteria grow best at a neutral to slightly alkaline reaction, i.e. pH 7.0–7.5, though some species are quite tolerant of acidity. So montmorillonite clays, at least at the higher water potentials needed for bacterial activity, are especially favourable for rapid development of bacteria whenever energy substrates become available. This high potential activity of bacteria in montmorillonite clays eventually provided a satisfactory explanation for the distribution of severe occurrences of the Panama disease of bananas in Central America and Jamaica; this disease is a vascular wilt (see Ch. 9) caused by the fungus *Fusarium oxysporum* f. *cubense*. O. A. Reinking (1935) first drew attention to the fact that severe and widespread outbreaks

of this disease in Central America were largely confined to light-textured sandy soils. From a later survey, C. W. Wardlaw (1941) concluded that both lightness of soil texture and some degree of acidity (often associated) favoured development of Panama disease; bananas growing on clay soils, especially if slightly alkaline, suffered much less from this disease. A similar distribution of the disease in Jamaica was noted by Rishbeth (1957), who also found that bananas often remained healthy, in an otherwise disease-devastated area, on sites of former sugar-cane factories. On such sites, sugar-cane trash (stem and leaf residues) had been dumped or burned for many years, so that the soils had an extremely high status in phosphate and potash. During my own stay in Jamaica during 1948, I had organized the setting-up of a series of field experiments, involving application of lime, in various combinations with N, P and K fertilizers, on areas infested with *F. oxysporum* f. *cubense*. These experiments were tended and the results were finally assessed by Rishbeth and Naylor (1957). Their results were scientifically interesting, insofar as Panama disease was added to the growing list of vascular wilts known to be aggravated by application of nitrogenous fertilizers. But our hopes for some degree of manurial control of this disease were dashed, because there was no significant response in disease reduction from applications of lime, phosphate or potash. So it seemed unlikely that field resistance of the banana to Panama disease could be effectively increased by any practicable fertilizer regime.

Some 10 years later, it began to seem probable that the clay soils unfavourable to disease development, which are known as "disease suppressive" or "long life" soils, might owe this character more to some effect in suppressing infection of banana rootlets than to a manurial effect in increasing disease resistance, as postulated earlier. These new ideas have been established by the extensive work of G. Stotzky and his associates on the distribution of Panama disease in Central America. Thus in a survey of 67 banana soils, Stotzky and Martin (1963) discovered a close correlation between long-life soils and the occurrence of a sufficient proportion of montmorillonite clay in the soil analyses. Stotzky and Rem (1966) studied the effect of adding graded amounts of montmorillonite, up to 5 % (*w*/*w*) to various species of bacteria growing in liquid culture; the clay much increased growth of the bacteria, estimated by rate of oxygen uptake in respiration. This effect was traced to the buffering effect of the clay particles, which stopped development of acidity in the cultures. This

approach to the problem was highly original, and conclusions were confirmed by further work. So Stotzky has concluded that the high level of bacterial activity permitted by montmorillonite-clay soils interferes with and restricts infection of banana rootlets by *F. oxysporum* f. *cubense*, and thus exerts a natural biological control of Panama disease in these long-life soils. No doubt the somewhat restricted aeration characteristic of moist clay soils also helps to reduce root infection by the pathogen, as noted above for the take-all fungus. Nor does this more recent work necessarily invalidate Rishbeth's (1957) conclusion that the usually high fertility of long-life soils, especially with respect to potash, helps the banana plant to resist development of this disease. Different hypotheses to explain a set of established facts are often assumed to compete on an "all or none" basis; such a rule is helpful to controversy but ignores the usual outcome of such controversies, which is that no-one has been wholly right and no-one completely wrong.

CHAPTER 3

LIVING INHABITANTS OF THE SOIL

In this chapter, we shall survey the various groups of organisms that inhabit the soil, beginning with the root systems of higher plants, on which all life in the soil ultimately depends. A brief account of the soil fauna will then be followed by a survey of the main groups of soil micro-organisms. Few research workers can afford the time to acquire and maintain a thorough working knowledge of all these groups of organisms, but all workers must know enough to recognize a development demanding detailed knowledge of a group of organisms other than their own, and thus to make the most of a new opportunity.

ROOT SYSTEMS OF HIGHER PLANTS

Plant root systems are the most important inhabitants of the soil, because directly by exudation of energy nutrients, and indirectly through the part they play in maintaining the shoot system (which also eventually dies and decays), they provide the energy that drives the whole of the soil ecosystem. This statement holds true both for areas under natural or semi-natural vegetation and for those under agricultural, horticultural and sylvicultural crops.

A discovery of outstanding interest both for soil microbiology and for agricultural science in general was made by the German microbiologist, L. Hiltner (1904), when he found that the young and active parts of root systems were surrounded by a zone of greatly enhanced microbial activity, which has become known as the *rhizosphere*. Numbers of soil bacteria in the rhizosphere may be ten times or more as high as those in soil remote

from roots. This is because young and physiologically active parts of the root system continuously exude energy nutrients, chiefly sugars and amino acids; it is these root exudates that support the rhizosphere micro-flora of bacteria, actinomycetes and fungi. For many years, this loss of organic nutrients by the root system remained somewhat of a mystery. J. L. Harley (1973) drew attention to the loss of energy by the forest eco-system through respiration of root exudates by soil micro-organisms. This effect of root exudates is obviously strongest at the root surface (the *rhizoplane*) and shades off gradually to the furthest limit of exudate influence in the rhizosphere. In recent years, however, microbiologists have abandoned the view that exudation of organic nutrients by roots indicates a degree of inefficiency in the mechanism for absorbing mineral nutrients from soil. We now know that many soil micro-organisms can extract mineral nutrients, especially phosphates, from soil more efficiently than can the roots of plants. The widespread occurrence of the *mycorrhizal symbiosis* between roots and various species of fungi in the root systems of the great majority of higher-plant species, indicates the efficiency of such mycorrhizal fungi in absorption of mineral nutrients from soil; a comprehensive account has been given by J. L. Harley (1969) in the second edition of his book, *The Biology of Mycorrhiza*. For the support of the specialized fungal sheaths around the ectomycorrhizal rootlets of forest trees, Harley (1978) has calculated that perhaps as much as 10 % of the tree's total carbohydrate production may be diverted to the ectomycorrhizal rootlets. There is no doubt that the tree's investment of part of its energy income in its fungal partner gives a profitable return in infertile soils, and especially in phosphate-deficient soils. Phosphatic fertilizers added to soil soon become converted into more or less insoluble forms that are not easily available to roots, though they may still be taken up by at least some micro-organisms. Losses of nitrogen from the forest ecosystem are much smaller, and they can be reduced to a minimum even in plantations. In many field trials on soils lacking both soluble phosphate and suitable mycorrhizal fungi, inoculation with such fungi has generally resulted in a significant and often very striking increase in rate of tree growth. Most plant species other than forest trees are regularly infected by fungal species belonging to another group (*endotrophic*) known as the vesicular–arbuscular group of mycorrhizal fungi. B. Mosse (1973) has summarized a growing body of evidence from field trials that, in phosphate-deficient soils, mycorrhizal infection by

these fungi increases phosphate uptake by their host plants and so gives valuable increases in crop growth and yield. The contribution made by these fungi to soil fertility is naturally more satisfactory to soil mycologists than is the more negative task of reducing loss of crop yield due to pathogenic root-infecting fungi.

An interesting discussion of how rhizosphere micro-organisms, especially bacteria, may increase the solubility of some plant nutrients has been provided by A. M. Smith (1976). He has pointed out that vigorous growth of bacteria on root exudates can create at least temporary and localized anaerobic micro-sites adjacent to roots. In such micro-sites, iron, manganese and some other trace-nutrient elements, that are present in the highly oxidized form, will be reduced to more soluble forms that can be taken up by roots. Similarly, soil-bound insoluble phosphate will be reduced in such anaerobic micro-sites to the soluble ferrous phosphate. Because this conversion from insoluble to soluble forms of these mineral nutrients is due directly to exudation of organic nutrients by active, younger parts of the root system, so these minerals will be released where they are most needed, i.e. adjacent to the surface of young and active roots.

THE SOIL FAUNA

Animal ecologists generally recognize three size-groups among the soil fauna: the micro-, meso- and macro-fauna. Amongst the micro-fauna, the large and varied group of Protozoa, which used to be classified in the Animal Kingdom, is now more properly assigned to the third or Protist Kingdom of micro-organisms (see next section). Members of the meso-fauna are defined as being too small, even when mature, to create additional soil space by burrowing, and are hence restricted to existing pore spaces. Many of them are known as micro-arthropods (belonging to the phylum Arthropoda, meaning "with jointed feet"). Particularly abundant in soil, and even more so in surface litter, are the mites with eight legs each (subdivision Acari of the Arachnida) and the springtails with six legs each (subdivision Collembola of the Insecta). Different members of this large and varied group feed on various kinds of plant and animal tissue, usually dead but occasionally alive, and also on soil micro-organisms and animal faeces. Their own faeces contribute a sizeable fraction of the degraded

organic material (humus) present in the soil, especially in forest soils. Also best included in the meso-fauna is the large group of eelworms (Nematoda); their slender acellular bodies vary in length, according to the species, up to *ca.* 0.5 mm, but some species grow to a length of 2.5 mm. Some species are saprotrophic, i.e. living on dead plant or animal tissues, others are predacious on smaller organisms, and yet others are of great economic importance as parasites of crop plants and domestic animals.

The macro-fauna include such well known organisms as those in the group Myriopoda (with many legs), which comprise both the millipedes (saprotrophic) and the centipedes (predacious on other organisms); these are commoner in the surface litter than in the soil itself. More economically important are larvae of the larger insects, some of which are troublesome pests of crop plants. But the most widespread and important organisms in the macro-fauna are the earthworms; all the earthworms of north-western Europe, including some twenty-five British species, are classified in the family Lumbricidae; species vary in length from *ca.* 2.5 to 25 cm. All of them live on dead plant refuse which, generally mixed with soil, is eaten and passed through the digestive tract, emerging as "worm casts" at the other end. Worm-cast soil is finely granular, with a rather stable crumb structure, and has a higher content of residual organic matter than soil not thus processed. Some species of worm typically come up to the soil surface to excrete their casts, and their continued activity tends to create a layer of finely granular, stone-free soil at the surface. Charles Darwin (1881), in his book, *The Formation of Vegetable Mould through the Action of Worms*, was the first to draw attention to this and other effects of worms upon the soil. But enjoyment of his first sight of the ancient monument of Stonehenge was somewhat clouded by his failure to find there as much evidence of the earth-moving activity of worms as he had hoped. His wife Emma remarked that "They did not find much good about the worms, who seem to be very idle out there".

The chief contribution made by earthworms to soil fertility is by their incorporation of plant residues lying on the soil surface with the mineral body of the soil underneath. The dead leaves that litter a woodland floor in autumn and early winter are dragged down into the soil by worms, and there devoured. In woodlands on neutral or alkaline soil, the large population of worms has disposed of most of the leaf litter by spring time;

the distribution of organic matter throughout the soil profile produces a *mull* type of woodland soil. But worms are intolerant of soil acidity, and their population declines markedly in numbers as acidity increases; the limiting soil pH value for worms is *ca.* 4.5. Worms need a continuous supply of calcium, which is secreted by glands along the digestive tract and helps to prevent too high a degree of metabolic acidity developing. Some factor in acid soils also acts as an irritant to the surface covering of worms; if worms are placed on the surface of too acid a soil, they do not burrow in as usual but remain on top of the soil and soon die. The absence of worms from acid soils results in a profound difference between the profile of such a *mor* woodland soil and that of a *mull* woodland soil, which is neutral or alkaline and therefore supports a large population of worms. In a mor soil, the plant litter is not incorporated with the soil but remains as a quite distinct layer on the surface; there it is decomposed *in situ* by soil micro-organisms and especially by fungi, which are more tolerant of acidity than are most bacteria. But decomposition of the litter layer is slow, because micro-organisms cannot get sufficient mineral nutrients (N, P, K, etc.) or sufficient basic ions to neutralize development of metabolic acidity.

The development of these mor soils is characteristic of the cold-temperate zone of climate that stretches from North America across north-western Europe and the U.S.S.R.; the climax community is coniferous forest. The soil profile is surmounted by a layer of partially humified leaves and twigs, always distinct and sometimes quite thick. Below this comes a layer of mineral soil that has been strongly leached by the action of humic acids carried down by percolating rain-water from the litter layer above it; little except particles of silica may remain in this layer, and so it is grey or even white in colour. Below this first layer of soil comes a second one, in which the mineral salts dissolved from the first layer have been deposited; this layer is usually rich in compounds of aluminium and iron, and the latter impart a reddish-brown colour to it. If this second layer has become bound together by the accumulation of salts, it becomes difficult for roots to penetrate and is then called a *hard-pan*. Such a soil profile, which is characteristic of soils under acid heathland as well as under coniferous forest in the cold-temperate zone, is known as a *podsol* (podzol); this is a Russian word meaning "ashes beneath", by reference to the ash-coloured layer of leached soil below the litter layer.

The evolution of such a podsol demonstrates the profound change in soil profile that results from the development of a degree of soil acidity too great for earthworms to tolerate; such acidity also reduces activity of the rest of the soil fauna. The effects of this restriction shows how great a contribution is made by the soil fauna in general, and by earthworms in particular, to maintenance of soil fertility by incorporation of plant refuse with the mineral body of the soil. The reduction of plant tissue into small fragments accelerates cellulose breakdown by bacteria in the digestive tract of the soil animals, and so the soil fauna and microflora co-operate in decomposition of plant residues and eventual liberation of nutrients in a soluble form for uptake by plant roots.

The only mammal that lives entirely within the soil is the mole (*Talpa europaea*), which is well adapted to burrowing through soil by its short but very powerful fore-legs; contrary to popular belief, it has functional though small eyes, unlike its prey, the earthworm. The ramifying system of "mole runs" in the soil is marked out by small heaps of excavated earth (mole hills) brought up to the surface. This system of burrows, like the narrower ones made by earthworms, contribute to soil aeration and drainage, but this contribution by the mole to health of the soil is not usually appreciated by the owners of grass lawns, bowling greens and tennis courts. Because the mole feeds chiefly on earthworms, it is usually absent from soils that are too acid for worms to tolerate. This fact has been memorably illustrated by G. V. Jacks (1954) in his book *Soil*. These observations concerned a grass tennis-court overlying an acid, sandy soil; Jacks noted that every spring-time, after the winter rains had washed away the powdered chalk used for marking out the white lines, the positions of all the former lines were delineated by straight runs of mole hills. Jacks concluded that this sandy soil was too acid for earthworms to thrive in it, except where the acidity had been sufficiently reduced by washing down of powdered chalk from the white lines. So the moles, themselves unseen, had through their workings indicated quite precisely the location of their prey, also unseen by the human observer. This is not only an example of the scientific mind at work to explain what was, in the first place, a chance field observation; it also epitomizes the indirect way in which soil scientists have to gather much of their information, because soil is an opaque medium and what goes on inside cannot be seen except by special techniques designed for the purpose.

SOIL MICRO-ORGANISMS

Classification

The first proposal that micro-organisms should be classified as a third kingdom, separate from the Plant and Animal Kingdoms, was made by Ernst Haeckel in Germany in 1866. Haeckel was a fervent disciple of Charles Darwin and well at the centre of the German Darwinian controversy, where he was the counterpart of Thomas Henry Huxley in Britain; his compaign is vividly described by William Irvine (1955) in his *Apes, Angels and Victorians*, a book that is as remarkable for its penetrating biological insights as for its scientific and social history of those times. The *Protists*, as proposed by Haeckel, took over the Protozoa from the Animal Kingdom, and the Algae, Fungi and Bacteria from the Plant Kingdom. The Protists as a whole were distinguished by their relatively simple organization and general lack of complex tissue differentiation; the chief exceptions to this generalization are to be found among the larger marine algae, and in the fruit bodies (toadstools) of the higher fungi (see Ch. 4).

It is rather difficult to explain why Haeckel's disposition of micro-organisms in this third kingdom, though well founded, took a whole century to gain general acceptance; we can define "acceptance" as inclusion in introductory texts, and I have to admit that there is no mention of protists in the first edition of this one (1963). But it seems that Haeckel was rather too vigorous a campaigner for the Darwinian cause; as William Irvine (*loc. cit.*) has said, he lacked Darwin's disarming tact in the presentation of new and alarming ideas. Among German biologists, it became the fashion to abuse Haeckel but to leave Darwin himself alone. So the general reluctance to embrace Haeckel's proposal of the protist kingdom may have been due largely to its undiscriminating advocacy by Haeckel himself.

The change of mind came from around 1950 onwards; more general use of the transmission electron microscope, and concurrent advances in comparative biochemistry and molecular biology, led to dramatic advances in our knowledge and understanding of the fine structure and functioning of plant, animal and microbial cells, as well as that of viruses; units of fine structure are measured in nanometres (1 nm = 10^{-3} μm = 10^{-6} mm). So work over the last 30 years has completely justified

Haeckel's proposal of the protists as a third kingdom of living organisms. It has also led to recognition of a fundamental distinction between two basic types of intracellular organization within the Protist Kingdom. These two types of cell are designated as *eukaryotic* and *prokaryotic*, respectively; the prefix "eu", common in biology, means "true" or "typical", and the prefix "pro" here means "earlier", i.e. a precursor of. All higher plants and animals, and the protozoa, algae and fungi among the protists, are eukaryotic; only the bacteria (protists) are prokaryotic. The intracellular organization of the prokaryotic cell is so fundamentally different from that of the eukaryotic cell that all prokaryotes are obviously more closely related to one another than they are to any group of the eukaryotes. This is why the group of Bacteria, as now recognized, includes taxa originally thought to be quite distinct. Thus the photosynthetic Blue-green Bacteria were for many years thought to be algae, and were called "Blue-green Algae" (Cyanophyceae). Similarly, the Actinomycetes, typical genera of which produce a true mycelium like that of the Fungi though much more slender, were originally thought to have affinities with the Fungi, as the termination "mycetes" implies. These explanations may help to clarify the modern classification of living organisms (Table 2).

TABLE 2. GENERAL CLASSIFICATION OF ORGANISMS

Plant Kingdom	Animal Kingdom	Protist Kingdom
(*Eukaryotes*)	(*Eukaryotes*)	*Eukaryotes*
Seed-bearing plants	Vertebrates	Algae
Angiosperms	Invertebrates	Protozoa
Gymnosperms		Fungi
Ferns		*Prokaryotes*
Mosses		Bacteria
Liverworts		(including Blue-green Bacteria and Actinomycetes)

The eukaryotic cell

The eukaryotic cell is bounded by the *cytoplasmic membrane*, seen under the electron microscope as a triple-layered structure of width *ca.*8 nm; such structured membranes are termed *unit membranes* and the eukaryotic cell

contains a variety, structurally distinct, of unit-membrane systems. These function to separate the wide variety of enzyme-mediated biochemical reactions that is proceeding concurrently within the cell. Passage of materials between one region and another is controlled by membrane transport. Most of the internal space of the cell is traversed by the endoplasmic reticulum (ER), which forms a network of membrane-delimited channels. Part of the ER forms the *nuclear membrane*, a distinctive structure perforated by many pores, of diameter *ca.*40 nm. The remainder of the ER is known as the *rough endoplasmic reticulum*, because it is coated with *ribosomes*, which mediate protein synthesis; the proteins thus produced are transported within the channels of the ER. Proteins and other materials synthesized on the ER are packaged for transport by the *Golgi apparatus*, which consists of a densely packed mass of sacs.

Two types of membrane-bounded *organelle* are responsible for respiration and photosynthesis, respectively. The *mitochondrion* (plural = mitochondria) carries on its internal membranes (*cristae*) the electron-transport system for respiration; the internal membranes (*thylacoids*) of the *chloroplast* carry the photosynthetic pigments (chlorophylls, etc.), the electron-transport system and the photochemical reaction centres. A quantitatively minor but nevertheless important fraction of the cellular genome is located in the mitochondrion and (in photosynthesizing eukaryotes) in the choroplast. This organellar DNA (Deoxyribonucleic acid) is seen as a small, double-helical molecule of circular form (i.e. an endless loop, usually much folded). These circular molecules are very similar to the circular DNA molecules of the prokaryotic cell, shortly to be described, though much smaller. This organellar DNA and the organelle containing it replicate themselves by division. The ribosomes concerned in the machinery of transcription and translation of organellar DNA resemble, both in size and in sensitivity to antibiotics like chloramphenicol, the 70S type of ribosome found in prokaryotic cells rather than the larger, 80S type of ribosome in the cytoplasm of eukaryotes. These resemblances of the organelles to whole prokaryotic cells furnish evidence for the idea that such organelles may have been derived from free-living prokaryotic cells that originally entered the eukaryotic cell and became established there as endosymbionts.

Replication and division of chromosomal DNA at nuclear division (*mitosis*), preceding division of the eukaryotic cell, has long been familiar to

biologists, because much of it can be clearly seen, after appropriate staining of cells, under the light microscope. The *chromosomes* thus visible from the beginning of mitosis are tightly coiled DNA molecules with which are associated both histone and non-histone proteins; they are visible under the light microscope because they have been much fore-shortened and thickened by coiling in preparation for mitosis. In eukaryotic cells, the number of chromosomes is always more than one, and both number and individual shape of chromosomes at mitosis are characteristic for the particular species of organism. At mitosis, a bipolar, spindle-shaped structure of *microtubules* is organized equatorially across the shorter axis of the cell. After alignment in the equatorial region of the mitotic spindle, each chromosome splits lengthwise into two *chromatids*. Finally, one complete set of chromatids moves to each pole of the mitotic spindle; the plane of cell division, separating the original cell into two daughter cells, is along the equatorial region of the spindle.

In the eukaryotic cells of vascular plants, algae and fungi, the cytoplasmic membrane bounding the protoplast is enclosed within a rigid cell-wall, which acts as a corset to the cell; thus it restricts uncontrolled expansion of the protoplast when the cell or tissue is immersed in a hypotonic liquid medium, of osmotic strength less than that of the cell sap. In higher plants, the cell wall (secondary) has a framework of cellulose, which in some tissues becomes further strengthened by deposition of lignin; the framework of the cell wall in some fungi is also of cellulose, but in the majority it is strengthened by chitin. So the function of the rigid cell-wall is control of protoplast expansion through turgor pressure. But in most of the protozoa, the cytoplasmic membrane is not bounded by a cell wall; this would prevent them engulfing bacteria and other microscopic cells that are their commonest prey in the soil. So osmoregulation in these protozoa has to be effected in another way, by means of the *contractile vacuole*. This functions as a cellular pump, which collects water from within the cell and periodically discharges it into the surrounding liquid medium, by coalescence with the cytoplasmic membrane (*exocytosis*). In the reverse process of *endocytosis*, molecules too large to pass through the cytoplasmic membrane and larger objects, such as cells of bacteria, are taken into the protozoan cell by an infolding (with some extension growth) of the cytoplasmic membrane, so that the object to be taken in becomes enclosed in a food vacuole. For digestion of the prey, food vacuoles fuse with

lysosomes, which are membrane-bounded vesicles (produced by the Golgi apparatus) containing a varied array of hydrolytic enzymes. In metazoan animals, this process of engulfment by endocytosis serves another purpose, that of defence against invading pathogens, and is then termed *phagocytosis*. In the human blood-stream, for example, two types of blood-cell predominate; the red cells bearing the oxygen-carrying haemoglobin, and the white cells, or *phagocytes*, which engulf bacteria and other invading pathogens. The idea of phagocytosis as a defence mechanism was first produced by Elie Metchnikoff (and further developed in the 1880s and later) while he was observing, under his microscope, the mobile cells of a transparent starfish larva; Metchnikoff's own account of this discovery is quoted (p. 69) by W. I. B. Beveridge (1950) in his *The Art of Scientific Investigation*, a book that can be recommended for anyone contemplating a career in biological research.

The prokaryotic cell

The organization of the prokaryotic cell, as at present understood from studies of its fine structure, appears to be simpler than that of the eukaryotic cell. In the great majority of prokaryotes, the cytoplasmic membrane is the only unit-membrane system of the cell, though it is made more extensive by much infolding. Only in the blue-green bacteria is there a second unit-membrane system; in these organisms, the photosynthetic pigments and apparatus are located on a series of flattened, membranous sacs, which are distributed through the cytoplasm. So the cell of a blue-green bacterium could be regarded as homologous with the chloroplast of a eukaryotic cell, if we accept the idea (mentioned in the previous section) that the chloroplast may have evolved from a photosynthetic prokaryote that had become endosymbiotic in a eukaryotic cell.

Electron micrographs of prokaryotic cells show the finely granular appearance of the cytoplasm, due to its content of ribosomes, each *ca.*10 nm diam.; they are of the 70S type and similar in size to the organellar ribosomes in eukaryotic cells. The protoplast is enclosed by a rigid cell-wall, which contains a substantial proportion of *peptidoglycan*; this class of wall polymer is *unique* to prokaryotes. The nucleoplasm, which can be seen after appropriate staining under the light microscope, is of irregular contour and is not bounded by a nuclear membrane; electron micrographs

reveal a fibrillar texture of the nucleoplasm, due to the double-helical strands (*ca.*25 nm wide) of DNA. The cytoplasmic membrane of the prokaryotic cell constitutes a barrier more selective than that of the eukaryotic cell, but it will permit passage of fragments of transforming DNA and of proteins of low molecular weight, such as exocellular enzymes produced by the cell. Hydrolysis of insoluble carbohydrates, such as cellulose, can take place only *outside* the cell, whether the enzyme remains bound to the cytoplasmic membrane or is set free for diffusion in the ambient liquid medium.

Most of the genetic information (*genome*) of a prokaryotic cell is carried on a *single, circular chromosome*. It is thus homologous not with the assemblage of chromosomes in a eukaryotic cell, but rather with the circular DNA molecules in mitochondria and chloroplasts. The length of double-stranded DNA in the prokaryotic chromosome varies up to a maximum of *ca.*3 mm (in some blue-green bacteria), whereas in most eukaryotic cells the length is *ca.*50 mm and sometimes more. At nuclear division, separation of the two daughter chromosomes appears to be effected by their attachment to specific sites on the cytoplasmic membrane, followed by extension growth of the membrane. During the exponential growth of bacteria on a substrate, nuclear division often runs ahead of cell division, so that single cells may temporarily contain more than one chromosome.

Genetic recombination in the prokaryotic cells of bacteria is a complex subject, and so will be described here only in outline. Three distinct types of genetic interaction are known. In *transformation*, a short length of double-stranded DNA released into the growth medium by a donor cell is taken up by a recipient cell and passes through its cytoplasmic membrane, with eventual degradation of one strand. In *conjugation*, a longer single strand of DNA passes from the donor cell into the recipient cell, with which it has temporarily fused by a conjugation tube. In *transduction*, a short, double-stranded fragment of DNA is carried from the donor cell into the recipient cell by an infective propagule of a bacteriophage (bacterial virus). Whichever the means of genetic transfer, the sequence of subsequent events is broadly similar. If genetic recombination does follow, it takes place by *exchange* between the complete haploid genome of the recipient cell and usually only a fraction of the donor genome. The haploid state of the recipient cell is soon restored by rejection of supernumerary fractions of DNA.

Genetic information additional to that borne on the bacterial chromosome may also be carried on *plasmids*; these are small, circular molecules of double-stranded DNA, with a molecular weight ranging around 6×10^7. Since the amount of DNA needed to code for an average polypeptide is about 6×10^5, some plasmids may contain as many as 100 genes. In a favourable cell environment, plasmids are autonomous, i.e. they reproduce themselves independently. If conjugation occurs naturally, or is induced experimentally, between cells of different species (or even of different genera) of bacteria, and if the recipient cell provides a favourable environment for a new type of plasmid from the donor cell, then the recipient cell will acquire, and pass on to its progeny, a new block of plasmid-carried genes. Thus the *R* factors for resistance to certain drugs and antibiotics are plasmid-borne, and have presented a difficult problem to medical research workers; for instance, plasmid-borne *R* factors carried by the bacterium *Escherichia coli*, which is normally a beneficial inhabitant of the human intestines, can be transferred by conjugation to bacterial pathogens causing dangerous enteric diseases, such as infective bacterial dysentery and cholera. Although nothing can stop the gradual, natural spread of *R* factors among bacterial populations, the widespread and indiscriminate misuse of antibiotics has been chiefly responsible for the appearance of bacterial pathogens with complete resistance to as many as five or more antibiotics and antibacterial drugs. Effective international legislation is now the only action that can reverse this trend.

Those wishing to learn more about the nature and functioning of eukaryotic and prokaryotic cells, and about the genetics and behaviour of micro-organisms, cannot do better than consult the 4th edition (1977) of *General Microbiology* by Stanier, Adelberg and Ingraham. This has been my principal source of reference for the writing of this section and the one preceding it. Also recommended is a review by M. J. Carlile (1980), "From prokaryote to eukaryote: gains and losses".

Soil algae

Algae are more common in soil than is often realized, at least in the surface layer, though which sufficient light for photosynthesis can penetrate. Their isolation from depths greater than this can usually be ascribed to the washing down of resting propagules by rain, or their downwards carriage by soil fauna. At least ten species are quite common in

soil, distributed among the Chlorophyceae (green algae), Xanthophyceae (yellow-green algae) and Bacillariophyceae (diatoms, which are silica-encrusted). Algae are mostly protected only poorly against desiccation and so one would not expect to find them in deserts, except as components of xerophytic lichens. Much of the so-called desert area in Egypt supports a rich and varied flora of ephemeral annuals and xerophytic perennials including bushes and small trees. But in areas too arid for growth of any vascular plants, green algae can regularly be seen on the underside of large, translucent pebbles. This microhabitat provides enough light for photosynthesis but also affords sufficient protection against the sun; the sharp fall of temperature at night, so characteristic of desert climates, causes some condensation of water as dew.

Soil protozoa

The protozoa are all unicellular protists, with a preponderance of animal-like characters, and so were formerly referred to as micro-fauna. Some of them are obligate parasites, e.g. the trypanosomes, which cause such diseases as African sleeping sickness, transmitted through the bite of the tsetse fly. The non-parasitic forms, which are widely distributed in most soils, are classified into the Mastigophora, which are flagellate, with longitudinal cell division; the Rhizopoda, typically amoeboid though some can produce flagella, and dividing by binary fission of a $\pm$ isodiametric cell; the Ciliata, which are motile by means of many cilia, organized into a co-ordinated locomotor system. Cell division is always transverse. Amongst the Ciliata are the most highly evolved unicells known to biology; the genus *Paramoecium* has been much studied. This organism is propelled through a liquid medium by the co-ordinated, rhythmical beating of the rows of cilia extending from its surface. Another series of cilia line a groove along one side of the cell leading to a mouth opening, into which the cilia sweep small microbial cells and other food particles. Such particles are enclosed within a food vacuole, which follows a regular track around the cell, finishing up at the anal pore, whence undigested material is discharged. Osmoregulation is effected by means of two contractile vacuoles, one at either end of the elongated cell and each at the centre of a system of radial canals, which drain the surrounding cytoplasm. The two vacuoles discharge their contents through the cell membrane alternately.

The amoeboid forms in the Rhizopoda move over a surface by the extrusion of finger-like extensions of the flexible cell, which are termed *pseudopodia*. Some species live as parasites in the gut of animals, including humans, and cause the serious disease of amoebic dysentery. Soil amoebae are mostly predacious, living by phagocytosis of bacteria and other unicells. Of much interest to mycologists has been the report by K. M. Old (1977) that a giant soil amoeba, later identified as *Arachnula impatiens*, preys upon the conidia of *Cochliobolus sativus*, a fungus causing a widespread foot-rot disease of cereal crops. The large, dark-green conidia are protected by a melanized outer wall, which is considered to protect the conidium from attack by most other soil micro-organisms. Nevertheless, Old has shown that when the amoeba enfolds a conidium, it produces one or more annular depressions in its wall. Further action results in the removal of a disk, up to 6 μm diam., which may be temporarily ingested by the amoeba. The entire protoplasmic content of the conidium is finally removed and digested by the amoeba, leaving an empty shell behind; the whole process was found to take 4–6h.

Early in this century, Russell and Hutchinson (1909) proposed the hypothesis that protozoa were an important regulator of bacterial numbers in the soil, through their predation. Some species of bacteria are distasteful to protozoa, which may thus exercise a selective effect on the bacterial population. This is discussed by E. W. Russell (1973) in his *Soil Conditions and Plant Growth*, an invaluable source of reference for all soil scientists.

Soil bacteria

Characteristics

In size, bacterial cells are of the order $0.5-1.0(-2.0) \times 1.0-2.0(-8.0)$ μm, and they are thus the smallest unicells among free-living micro-organisms. Commenting on the small size of bacterial cells, Stanier *et al.* (1977) have referred to the biological generalization that rate of metabolism is inversely related to the size of an organism. As growth rate is largely determined by metabolic rate, small organisms grow faster than larger ones; a high growth rate confers two advantages. Firstly, many substrates in soil are ephemeral, especially sugars and amino-acids occurring in root exudates; bacteria commonly have a doubling time of less than one hour

under optimal conditions, and so high a growth rate enables bacteria to secure a large proportion of an ephemeral substrate in competition with fungi, at least when conditions favour their activity. Secondly, we can note that the high growth rate of a bacterial population, when substrate and environment permit, means an equally rapid production of genetic recombinants; this confers a maximum degree of genotypic flexibility on the bacterial population, and hence a flexible response to any change of environmental conditions within the soil habitat.

The range of morphology in bacterial unicells that can be distinguished under the light microscope is necessarily limited. Cells may be spherical (cocci) or cylindrical (rods); they can also take the form of short, curved rods (*Vibrio* spp.) or long, helical rods (*Spirillum* spp.). Some bacterial genera are non-motile; others are motile through the possession of *flagella*, which may be attached to one end of the rod-shaped cell (*polar*) or disposed all round it (*peritrichous*). Pioneer bacteriologists of the 19th century, and those following them, found this limited range of morphology among bacteria was inadequate to describe a newly discovered species with sufficient taxonomic precision. So morphological description had to be supplemented by the use of differential stains and other microchemical tests. Even more useful was the gradual evolution of a wide range of biochemical tests, involving tests of ability to decompose various kinds of carbohydrate and other organic compounds, together with by-products of the decomposition, such as evolution of gas and development of metabolic acidity. Nowadays various kinds of serological test and other techniques are available for identification of bacterial cultures and as an aid to taxonomic classification.

The most fundamental division of bacteria into two major taxonomic groups, *Gram-positive* and *Gram-negative*, is known as the *Gram-reaction*. Dried films of a bacterial suspension are stained and counter-stained on a microscope slide, by a method devised by Christian Gram in Denmark in 1884. Only some 20 years ago, however, was the Gram-reaction fully explained through differences in chemical composition and ultra-structure of the cell wall. In electron-micrographs of wall sections, the wall of a Gram-positive bacterium is seen as a uniform layer, 20–80 nm wide; that of a Gram-negative bacterium consists of a dense, rigid inner layer (2–3 nm wide) and a thicker outer layer (8–10 nm wide). In all bacteria, the wall polymers chiefly responsible for the tensile strength of the cell-wall belong

to the class of *peptidoglycans*; these are unique to the prokaryotic cell, as noted earlier in this chapter. Chemical analyses have shown that peptidoglycan is a major constituent (50–80 % by weight) of the wall in Gram-positive bacteria. In Gram-negative bacteria, peptidoglycan comprises 1–10 % total weight of the wall but is confined to the inner, rigid layer, which is a shell of almost pure peptidoglycan.

Biological activities of soil bacteria

In the final analysis, bacteria are essential for the maintenance of soil fertility, whereas fungi are not; the chief contribution made by fungi is through their rapid, pioneer colonization of plant residues, which accelerates decomposition and the ultimate mineralization of the major nutrients in a form available for uptake by higher plants, i.e. as nitrates, phosphates, sulphates and salts of potassium, magnesium and calcium, etc. For the synthesis of all protoplasm, whether of plants, animals or microbes, nitrogen is required in larger amounts than is either phosphorus or potassium; in soil supporting active growth of vegetation, nitrogen is the mineral nutrient that most commonly limits growth of both plants and micro-organisms. Most bacteria and fungi are able to decompose organic nitrogen compounds in plant and animal residues, with the ultimate liberation of ammonia. But ammonia can be oxidized to nitrate *only* by the nitrifying bacteria. Such bacteria are known as *chemo-autotrophs* and they obtain their energy by the exothermic oxidation of ammonia to nitrite, and of nitrite to nitrate. Substrate specificity within this group of chemo-autotrophs is high; no bacterial species can carry out both these oxidations in the conversion of ammonia to nitrate. Representatives of these two groups were first isolated by S. Winogradsky in 1890 by the expedient of using strictly inorganic isolation media; this was an outstanding technical feat at that time. The ammonia-oxidizer was given the generic name of *Nitrosomonas* and the nitrite-oxidizer that of *Nitrobacter*. At the present time, five species in four genera are known to be ammonia-oxidizers, and three species in three genera are nitrite-oxidizers (Stanier *et al.*, 1977). All species are Gram-negative, but there are wide differences in cell morphology between the species.

A second, important contribution to the nitrogen cycle is made by those micro-organisms that can fix atmospheric (molecular) nitrogen in the form of ammonium nitrogen. Ammonium-N can be taken up by all micro-

organisms except a few highly specialized parasites; for certain higher plants such as some species of conifer typically growing on acid soils, ammonium-N is a better source of nitrogen than is nitrate-N, whereas in less acid, or in neutral to alkaline soils, ammonium-N is quickly converted to nitrate-N. Molecular nitrogen is fixed only by prokaryotes, i.e. by bacteria, in the widest, present sense of this grouping. Claims for nitrogen fixation by fungi have been made, but it proved impossible to confirm them, and it now seems certain that this ability is limited to the prokaryotic cell. Nitrogen can be fixed (1) by bacteria growing symbiotically with the host plant in nodules on its root-system (2) by free-living bacteria. Much the most important source of fixed nitrogen is that produced by species of the Gram-negative genus *Rhizobium*; five species are recognized, largely on their host range within the Leguminosae. If the appropriate bacterial symbiont is absent from a soil because a particular legume species has not been grown in that soil, then the correct species of *Rhizobium* can be introduced by seed inoculation; thence it grows out along the surfaces of roots and the whole root system becomes nodulated. Root nodules are also regularly produced by some plant species in families other than the Leguminosae; the best known of these species is the alder (*Alnus glutinosa*). In some of these species, ability of the nodules to fix molecular nitrogen has been proved by use of radioactive-tracer nitrogen. Microscopical examination of the nodules has shown them to contain micro-organisms tentatively identified with the Actinomycetes, which are grouped with the Gram-positive Bacteria (see below). So far, however, attempts to isolate and culture the symbionts have failed.

By comparison with the weight of molecular nitrogen fixed per unit area of a leguminous crop (*ca.* 280 Kg N/ha), that fixed by free-living soil bacteria is much smaller. Blue-green bacteria of the genera *Anabaena* and *Nostoc* fix only about one-tenth the weight of nitrogen fixed by the same area of a leguminous crop. Nevertheless, the flooded fields of rice paddies in the tropics are an environment particularly suited to these blue-green bacteria, and their contribution to nitrogen-fertility is thought to be agronomically significant. Other free-living bacteria make a contribution to nitrogen fixation that is still smaller, about 1 % of that fixed by the blue-green bacteria. The best-known genera are *Azotobacter* (aerobic) and *Clostridium* (anaerobic). *Azotobacter* spp. can maintain themselves, on root exudates, in the rhizosphere of young and active roots and this fact

may account for the success claimed by soil microbiologists in the U.S.S.R. as a result of seed "bacterization" with *Azotobacter*. Increases in crop yield in some field experiments have also been obtained at the Rothamsted Experimental Station; the whole subject has been reviewed by Margaret E. Brown (1974), who has concluded that production of gibberellins, and possibly of other growth hormones too, by *Azotobacter* may be responsible for much of the increase in crop yield due to bacterization.

Soil actinomycetes

These micro-organisms are common in soils, especially in neutral and alkaline soils. At one time, their closest affinities were thought to be with the Fungi, as their name (meaning "ray fungi") implies. But the eventual discovery that the actinomycetes are prokaryotes has placed the order Actinomycetales firmly inside the general group of Bacteria, despite the fact that some of them produce a true *mycelium*, comparable with that of fungi except that the hyphae are much more slender (0.5–2.0 μm diam.). The actinomycetes are all Gram-positive, but in other respects form a rather heterogeneous group, as indicated by their subdivision into Proactinomycetes and Euactinomycetes. In the *Proactinomycetes*, mycelial development is transitory and limited; reproduction and dispersal occurs through fragmentation of the mycelium into short, rod-shaped cells. Closest affinities are with the coryneform bacteria, amongst which are dangerous human pathogens such as *Corynebacterium diptheriae* and *Mycobacterium tuberculosis*. In the *Euactinomycetes*, the most widespread and typical genus is *Streptomyces*, which grows entirely in the mycelial form. During active growth of a colony, the mycelium is mostly aseptate (coenocytic). As in fungi, growth occurs from the hyphal apices, and hyphal branches arise behind the main apex of a leading hypha in regular succession. A colony consists of mycelium immersed in the growth substrate and aerial mycelium above it; the aerial hyphae are surrounded by an additional wall-layer, or *sheath*, which resists wetting. Spores are produced by septation of the apical region of the aerial hyphae, and subsequent separation of the cells.

In other respects, actinomycetes are much like bacteria in general, and their colonies grow at much the same rate, more slowly than those of most fungi. Like the majority of bacteria, actinomycetes are usually intolerant of

acidity. This is well shown by the distribution of *Streptomyces scabies*; the scab of potato tubers that it causes is most prevalent on neutral and alkaline soils, and also on soils that are well aerated, i.e. light-textured sandy soils. The "earthy" odour of newly wetted soils has been traced to a volatile growth product of actinomycetes, and similarly an unwelcome taint at one time found in the flesh of freshly caught Scots salmon. But the actinomycetes, and especially *Streptomyces* spp., are most widely known for their production of a range of medical antibiotics. In 1943, Selman A. Waksman and his team of research associates at Rutgers University, N. J., discovered the antibiotic *streptomycin*, produced by a strain of *Streptomyces griseus*, use of which eventually ended the ravages of tuberculosis. This and other antibiotics are produced in pure culture only after vegetative growth of the producing micro-organism has virtually ceased; i.e. they are products of a mature culture and are hence termed *secondary metabolites.*

The discovery of streptomycin by Waksman and his team in 1943 was no accident, but rather one of the first fruits of a carefully planned programme for screening a large number of soil actinomycetes. For this venture, Waksman was uniquely qualified by some 30 years' work in soil microbiology, and by his international status as an authority on the Actinomycetes. After publication of his *Principles of Soil Microbiology* in 1929, he was regarded as the leading soil microbiologist of his time; his book still remains the best and most detailed account of work published up to 1930. Waksman's book was a great stimulus to those of us beginning in soil microbiology at that time; he will be long remembered for his kindly advice and encouragement to young research workers who sought his help, and not least by me.

CHAPTER 4

FORM AND FUNCTION IN FUNGI

The vegetative body of a fungus consists of a branching system of hollow tubes, at first filled with protoplasm; this is known as a *mycelium*, which grows both upon and within its substrate. The commonest substrate for a soil fungus is a corpus of plant tissue which may be alive (and can then, by definition, be colonized only by a parasite) but more usually is dying or dead. Such substrates may vary in volume from microscopic dimensions up to that of the roots and collar region (butt) of a forest tree.

ORGANIZATION AND GROWTH OF MYCELIUM

A mycelium is made up of individual, branching *hyphae*, usually of 3–10 μm diam. A fungal colony begins growth from some kind of propagule, most commonly a spore; in the soil, such propagules usually germinate only in response to a nutrient stimulus, such as root exudate or soluble nutrients from a corpus of fresh plant tissue. Part of the mycelium from such a young fungal colony is shown in Fig. 3.

Growth of a fungal hypha is made by the conical apex; there new protoplasm is synthesized and cell-wall is secreted around it. As shown in Fig. 3, the first hyphal branch is produced behind the growing apex, at a distance fairly characteristic for the fungal species. Branches of the first order produce branches of the second order, but an orderly hierarchy of development is maintained. Thus Gillian M. Butler (1961) found that, in the mycelium of *Coprinus disseminatus*, growth rates of leading hyphae, branches of the first order, and those of the second order, respectively, were in the ratio 100:66:18. Also to be noted in Fig.3 are the *septa* (cross-walls)

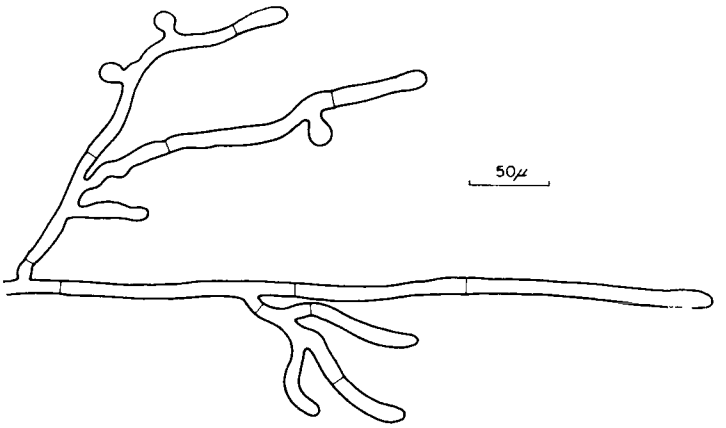

FIG. 3. Young mycelium of *Rhizoctonia solani*.

which divide the hyphae into compartments; such a mycelium is said to be septate. In the taxonomic class of fungi formerly named the Phycomycetes, the fungal mycelium (where formed; in some groups the fungal thallus is non-mycelial) is aseptate or *coenocytic*; septa are produced only for the delimitation of sexual organs or to seal-off a damaged or dying length of hypha. In present fungal taxonomy, the former large and unwieldy class of mycelial Phycomycetes has been divided into (1) the subdivision Mastigomycotina, which includes the class Oomycetes (2) the subdivision Zygomycotina, which includes the class Zygomycetes. A broad outline of present fungal classification will be given later in Table 4 (next chapter).

In the days of light-microscopy and early microchemical techniques, the fungal cell-wall appeared as a homogeneous single layer. Transmission electron-microscopy has revealed several wall-layers; in the much studied *Neurospora crassa*, there are four. The innermost layer is made up of a network of chitin micro-fibrils, some of which extend to several μm in length, intermixed with protein; next comes a layer of protein, surrounded by a third layer with a coarse reticulum of glycoprotein in a matrix of amorphous glucan with some protein. The outermost layer consists of amorphous glucans. These chemical data have been provided by various sophisticated techniques and especially by that known as "enzyme dissection". The mechanical, and especially the tensile, strength of the wall

is provided partly by the network of chitin micro-fibrils in the innermost layer, though the whole structure is interlocked by chemical complexing. Chitin is a polymer of N-acetylglucosamine, and is a wall constituent in the great majority of fungi. But the cell walls of aseptate fungi in the taxonomic class Oomycetes have long been known to contain no chitin and this was thought to be replaced by cellulose as a major structural component. Cellulose, however, is a straight-chain polymer with (1–4) linkages between the glucose units. The "fungal cellulose" of oomycete fungi is a branched polymer; the main chain has (1–4) linkages and the side branches are (1–3)-linked. So this fungal cellulose has a degree of crystallinity lower than that of true cellulose, but it can be hydrolysed by cellulase enzymes, so the biological difference between the two is not great. In his study of the antibiotic *griseofulvin*, P. W. Brian (1949) demonstrated dramatically this difference in fungal wall composition between species in the Oomycetes and others with chitinous walls. Griseofulvin was originally called the "curling factor", because it caused curling and distortion of growing hyphal tips in chitin-walled fungi. But not one of the oomycete species tested by Brian was sensitive to this action of griseofulvin, so he ascribed its effect to a dislocation of normal development and maturation of the chitin component of the hyphal wall. Griseofulvin has no toxicity to mammals and its antibiotic activity seems restricted to chitin-walled fungi; it has now become the best agent for control of fungal infections (*mycoses*) of animals and men.

A second fundamental distinction amongst fungi is provided by the ability of hyphae of septate fungi to fuse with others in the same colony or of the same species; such fusions are called *anastomoses*, and occur only when there is a meeting of hyphal tips, in the region where the hyphal wall is still plastic. No such anastomoses occur between vegetative hyphae of typically aseptate mycelia in the taxonomic sub-divisions Mastigomycotina and Zygomycotina; in these fungi, hyphal fusions are limited to those between male and female sexual organs. But in the septate fungi, hyphal fusions with significance for genetic recombination are not restricted to visibly differentiated sexual organs. Apart from their genetical significance, hyphal fusions may convert a mycelium into an anastomozing network, thus facilitating distribution and redistribution of nutrients in short supply. Thirdly, such hyphal fusions play their part in the interlocking construction of fungal tissues and organs, such as mycelial strands and

fruit bodies, to be described later in this chapter. So in the aseptate fungi, in which hyphal fusions do not occur, no true tissues and no true sporocarps are found. The nearest approach to the latter is found in the genus *Endogone* (Zygomycetes), in which a group of zygospores is invested by a tangled mass of loosely interwoven hyphae.

In septate fungi, each septum develops as a ring of wall material growing inwards from the innermost layer of the hyphal wall, in the manner of an iris diaphragm closing a lens aperture. Most commonly the septum does not completely seal off one hyphal compartment from the adjoining one, because a *central pore* is left open, at least for some time. Through this central pore can pass cytoplasm and its eukaryotic organelles (Ch. 3) and even nuclei. The pore is often just a simple perforation, though a rim develops in some genera. In the *dolipore*, characteristic of the Basidiomycotina (which includes the macro-fungi producing toadstools, fruiting brackets on trees, and puff balls, as well as others), the pore rim is extended into a deep flange, so as to produce a barrel-shaped pore. In general, we can conclude that septation of a hypha increases its mechanical strength and rigidity. It seems likely that eventual plugging of septa occurs when communication between the active region of a hypha and the older part is no longer needed.

Growth rate of fungal colonies

Fungal growth rate has been studied chiefly in the laboratory, with the fungus growing in pure, or axenic, culture. Growth on or in nutrient media has been studied (1) in liquid media (2) on media solidified by incorporation of a gelling agent, agar (Ch. 6). By the first method, a fungal colony is grown either in a stationary layer of liquid nutrient medium, shallow enough to permit adequate diffusion of oxygen and carbon dioxide, or, more usually, in a culture vessel aerated either by incubation on a mechanical shaker or by passage of a stream of sterile filtered air. Growth of the colony is assessed at intervals by the taking of replicate samples; the mats of mycelium are filtered off, washed and oven-dried to constant weight. By the second method, colonies are grown inside circular, closed Petri dishes (shown in Plate 1, Figs. 1 and 2) filled with nutrient agar to a depth of *ca.*3 mm. Colony diameters are measured at daily intervals. As an alternative to Petri dishes, horizontal glass tubes (1.5–2.0 cm diam.),

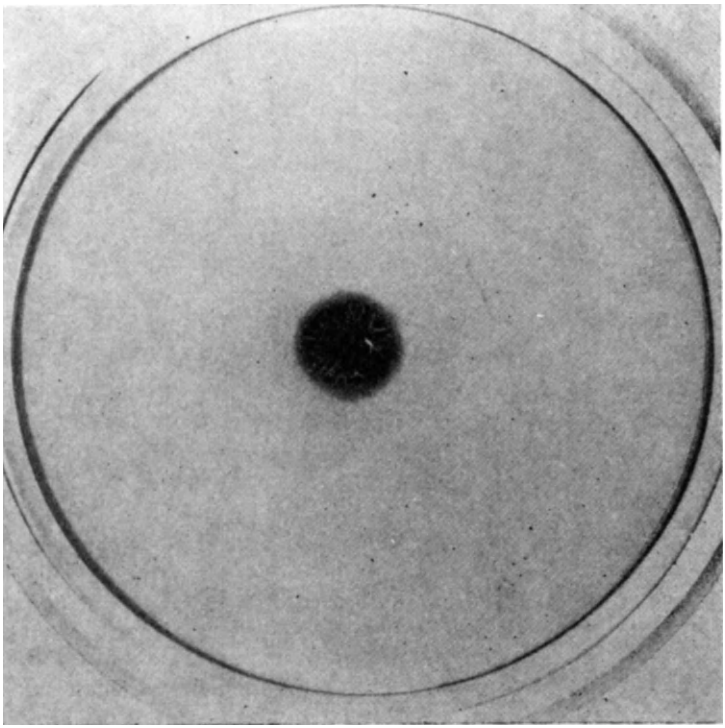

PLATE 1. FIG. 1. A circle of white rhizomorph initials has arisen behind the growing margin of a young colony of *Armillaria mellea* on nutrient agar (viewed from below).

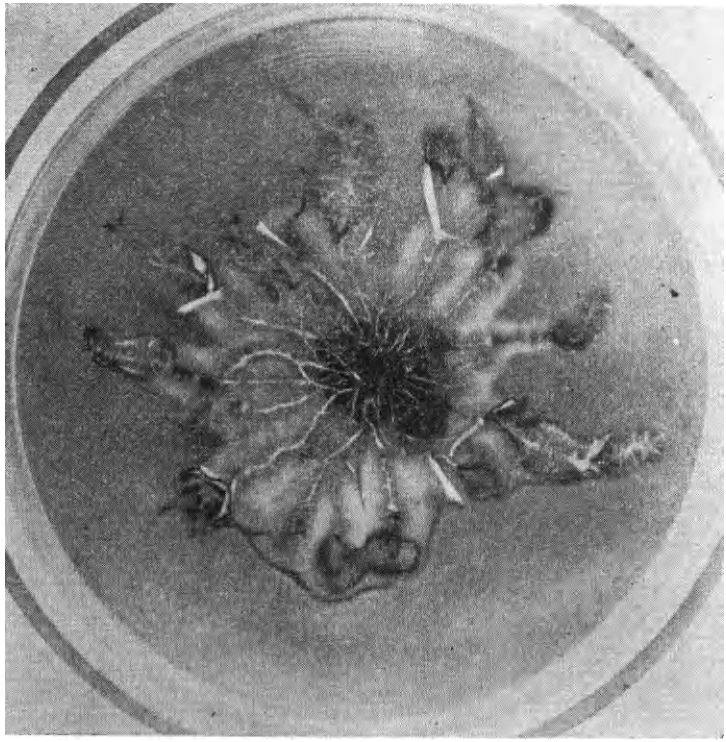

FIG. 2. The same colony 13 days later (viewed from below). The rhizomorphs have overtaken in growth the original mycelial margin of the colony. From these growing rhizomorphs and their branches fringeing mycelium has grown out, to give a fungal colony of characteristically lobed appearance (From S. D. Garrett (1953). By permission, *Annals of Botany*).

turned up at each end and plugged with cotton wool, can be used to contain the nutrient agar.

In the past, there has been some confusion as to which of these two methods has been more appropriate to employ for the study of a particular ecological situation. Measurement of radial, i.e. linear, growth rate of colonies over agar has often been chosen because it is much less laborious than measurement of mycelial dry weight. Choice should be based not on convenience, however, but on what we want to know. If we wish to compare the speed with which two or more fungi can cover a given distance, say from a resting propagule to a nearby root or a substrate of fresh plant tissue in soil, then measurement of growth rate over agar will be appropriate. But if we wish to explain differences in longevity of saprophytic survival between several fungi in plant-tissue substrates of a standard volume, then we will wish to know the relative speed at which the different fungi will consume the nutrients contained by such a standard volume of substrate. For this purpose, rate of increase in mycelial dry weight (as measured in liquid culture) will afford the better indication of *general metabolic rate* of the fungi being compared. Other things being equal, the time of survival for a fungal colony in a given volume of plant-tissue is likely to be *inversely* proportional to its general metabolic rate.

If growth rates are assessed by these two methods for a small group of similar fungi, both methods may give much the same result, i.e. the fungi will be arranged in the same order by either method. But if a larger group of more diverse fungi is studied by these two techniques, then discrepancies are likely to be found. The reasons for this have been explained by A. P. J. Trinci (1971) in an illuminating paper entitled "Influence of the width of the peripheral growth zone on the radial growth rate of fungal colonies on solid media". For a group of nine fungal species, Trinci compared *specific growth rate* of mycelial mass in liquid culture with radial growth rate of colonies on nutrient agar. Radial growth rate (Kr) over agar was shown to be determined partly by the specific growth rate (α) in liquid culture and partly by the width of the *peripheral growth zone* of the colony (w):

$$Kr = \alpha w$$

The peripheral growth zone was defined as that breadth of the marginal region of a colony over which protoplasm in the forepart of the leading

hyphae contributes to their rate of apical growth. The width of this zone was experimentally determined by making a razor cut behind the colony margin, so positioned that the leading hyphae were severed at various distances from their apices. The width of the zone was then taken as that at which severance just failed to reduce growth rate of the leading hyphae. So we can conclude that a certain volume of fungal protoplasm is necessary to maintain maximum growth rate by a leading hypha; this volume varies between fungal species. Within such a growth unit, movement of protoplasm and nutrients towards the hyphal apex appears to be effected by the process of *cytoplasmic streaming*; the occurrence of such streaming is limited to the distance from the apex backwards to the first completely plugged septum. But if the mycelium is coenocytic, or if septal pores in a septate mycelium remain open for a long distance behind the growing apex, then no such limitation is imposed. These facts help to explain the high radial growth rate over agar that is characteristic of fungi with coenocytic mycelia, in the classes Oomycetes and Zygomycetes.

Although radial growth rate over agar by itself is not a reliable guide to specific growth rate in liquid culture, yet the equation given above shows that there is a relationship between these two values. If we determine experimentally both radial growth rate (Kr) and width of the peripheral growth zone (w), then we can calculate specific growth rate (α) from the above equation transposed thus:

$$\alpha = Kr/w$$

From his experimental data, Trinci has calculated theoretical values of specific growth rate (α) for nine species of fungi and has then compared them with observed values obtained from growth rate of mycelial mass in liquid culture for the same species. The correlation coefficient (r) for the association between these two sets of values was found to be 0.9825, a value that is highly significant (at the 0.1 % level). The regression line drawn in Fig. 4, connecting these two sets of values given by Trinci in his Table 4, shows a close relationship between calculated and observed values of specific growth rate.

We now have all the information to explain why a random group of fungal species may fall into one order when arranged according to radial growth rate over agar, and into another when arranged according to rate of growth in mycelial mass. Growth rate of mycelial mass is determined by

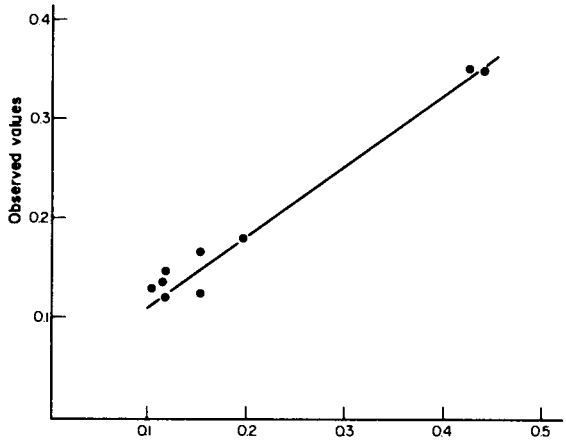

FIG. 4. Regression of observed on calculated values of specific growth rate (α) for nine fungal species, plotted from data provided by A. P. J. Trinci (1971) in his Table 4.

growth of the *whole mycelium*, i.e. leading hyphae, branches of the first order, and those of the second and further orders. As noted above, when citing the paper by G. M. Butler (1961) on *Coprinus disseminatus*, the growth of leading hyphae is faster than that of branches of the first order, which in turn is faster than that of branches of the second order, and so on. Precise values for this hierarchical order of growth rates differ between different species. This is why growth rate of the leading hyphae in a colony on agar cannot be used, by itself, to predict rate of growth in total mycelial mass.

Translocation of nutrients through fungal mycelia

The quickest way in which nutrients can travel through a coenocytic hypha, or through septal pores in a septate hypha, is by mass movement of cytoplasm, by the process of *cytoplasmic streaming*. A full discussion of possible mechanisms of translocation has been provided by J. H. Burnett (2nd ed., 1976), in Ch. 9 of his *Fundamentals of Mycology*; this book can be recommended to those who wish to learn more about matters discussed in

the present chapter and the next. Rate of cytoplasmic streaming increases with temperature up to the optimum and can exceed 20 cm/h; it tends to be higher in coenocytic hyphae but can be just as fast through septal pores in some species.

Impetus to studies of translocation was given by K. H. Schütte (1956) in a paper entitled "Translocation in the fungi". By use of simple techniques, Schütte showed that some fungi were able to translocate nutrients from a food-base through an established mycelium traversing a space devoid of nutrients, whereas other fungi were unable to do so. Later workers have used radio-active tracer nutrients in similar techniques. For the group of fungi that he studied, Schütte correlated translocating ability with the occurrence of cytoplasmic streaming. Later workers have confirmed Schütte's general conclusions, though some fungi of "indeterminate" translocating ability have predictably been reported. Nevertheless, the optimism of research workers usually triumphs over their past experience that biological boundaries are rarely as sharp as they seem at first to be.

This subject has been reviewed by R. L. Lucas (1977) in a paper entitled "The movement of nutrients through fungal mycelium", in which he has described experiments with the zygomycete *Rhizopus stolonifer*, using potassium dihydrogen phosphate labelled with 32-P as a radio-active tracer nutrient. Transpiration by the fungus was artificially increased by cutting holes in the Petri-dish lids; an increased transpiration was reflected both by precocious sporulation of *R. stolonifer* and by an increase in uptake of 32-P. Transpiration causes a loss of turgor pressure, especially in the apical regions of leading hyphae, which are less well protected against desiccation than are the older hyphal regions; this loss of turgor pressure is then equalized by forwards streaming of cytoplasm towards the hyphal apices. In such growing fungal colonies, translocation of 32-P was predominantly in a forwards direction, from the older mycelium towards the colony margin. In general, maximum uptake of 32-P occurred when a ventilating hole in the dish lid was situated over the growing margin of the colony *after* the mycelium had begun to take up the phosphate (compare Figs. 9 and 10 in Lucas's paper).

There is still argument about the precise mechanism of fungal translocation and various hypotheses have been discussed by Burnett (Ch. 9, 1976); most, if not all, the hypotheses may contain an element of the whole truth. But these experiments by Lucas have shown that, when the

transpiration rate of growing hyphae is increased, so is the rate of nutrient translocation; in this situation, translocation is mediated by forwards cytoplasmic streaming, which corrects the turgor-pressure deficit caused by transpiration at the hyphal apices.

ASEXUAL REPRODUCTION AND DISPERSAL OF FUNGI

Spores are the chief reproductive propagules of most fungi and serve two functions: dispersal in space and survival in time. The requirements for fulfillment of these two functions, as perfected by evolution, are usually incompatible, though some types of spore function efficiently for both dispersal and survival. For air-borne dispersal, spores must have a high volume/weight ratio and hence must be thin-walled; nevertheless, some such spores are still efficient survival-propagules. Many types of resting spore are thick walled and so unsuitable for air dispersal, though they can be dispersed by other agencies, such as rain splash, moving water, insects and other arthropods and through the digestive tracts of herbivorous mammals (the coprophilous fungi, fruiting on animal dung). Spores of all types can be produced either by a sexual process, or asexually. A sexual, or quasi-sexual, process provides opportunity for genetic recombination, whereas asexual spore production typically does not. Many fungi also produce another type of reproductive propagule: the *sclerotium*. This is an aggregation of fungal cells, usually isodiametric; specialized types of sclerotia usually have a differentiated outer layer (the *rind*) of smaller but thicker-walled cells, often darkened by deposition of melanin pigment. Melanization is thought to be both a response to and a protection against attack by other soil micro-organisms. Sclerotia function as resting bodies, i.e. organs specialized for survival, and sometimes can persist in soil for periods similar to those of long-lived seeds of flowering plants.

If a fungal mycelium is bearing sexually produced spores, it is said to be in the *perfect state*; if it is carrying only asexually produced spores, then it is in the *imperfect state*. In general, the range of substrate and environmental conditions permitting sexual reproduction is narrower than that sufficing for asexual reproduction. As in higher plants, so in fungi the characteristics of the sexual process are less labile than those of asexual propagation, and so the former provide the sounder basis for a natural classification, based on probable phylogeny (see Ch. 5, Table 4). Nevertheless, a more artificial

classification, i.e. a key for identification, is still required for the imperfect states of fungi. This provides for the description and naming of newly discovered imperfect states and for the recognition of those already established. Some 1500 of these genera, sometimes called "form genera", have been described and are known collectively as the Fungi Imperfecti (Deuteromycotina). Both in the natural environment and in the laboratory, asexual spores are produced more readily than sexual ones; so the imperfect state of a fungus has often been described and named before discovery of its perfect state and establishment of a connection between the two. So the Fungi Imperfecti comprise three assortments of species: (1) those for which connection with a perfect state has been established by observation and experiment (2) those for which a perfect state may be suspected but is not yet proven (3) those for which a perfect state has not been found. In this third group may be included some imperfect genera that do not produce a perfect state at all; the discovery of modes of genetic recombination other than the sexual cycle makes this quite possible (see Ch. 5). The great majority of imperfect fungi that have been paired with perfect states belongs to the Ascomycotina (still often referred to as the "Ascomycetes"). But a minority belongs to the Basidiomycotina (Basidiomycetes); the sterile mycelium of *Rhizoctonia solani* (Fig. 3), a widespread soil-borne pathogen of plants, has a perfect state (*Thanatephorus cucumeris*) belonging to the Basidiomycotina. An outline of the present classification of the Fungi Imperfecti is given in Table 3.

TABLE 3. OUTLINE CLASSIFICATION OF THE FUNGI IMPERFECTI (*DEUTEROMYCOTINA*)

Blastomycetes:	asexual states of yeasts and yeast-like forms
Hyphomycetes:	mycelia either sterile or bearing conidia on single hyphae or on loose aggregations of hyphae
Coelomycetes:	conidia produced (1) inside flask-shaped pycnidia (2) on cushions of crowded, parallel conidiophores (acervuli)

Dispersal of the asexual spores occurs in various ways, according to whether the spores are produced in a dry or in a slimy state. E. W. Mason (1937) proposed that a natural subdivision of the Fungi Imperfecti should

be based on this difference. Thus, all the Coelomycetes (Table 3) and some of the Hyphomycetes produce *slime spores* (Gloiosporae), whereas the remaining Hyphomycetes produce *dry spores* (Xerosporae). Dry spores are detached and liberated into the air by wind or mechanical shock; slime spores are aggregated into a head surrounded by mucilage and cannot be blown or shaken off their conidiophore. They are distributed through soil by moving water and above ground by rain splash. Some fungi produce their slime spores in a sugary medium like nectar, which is usually scented and so attracts the insects that disperse the spores. The human sense of smell is far inferior to that of insects and so the scent of this "nectar" may be either imperceptible by us or often unpleasant, e.g. that produced by fruit bodies of the stink-horn fungi (*Phallus* spp., the perfect state of a basidiomycete genus), which is attractive to flies. In Fig. 5, examples are shown of (*a*) slime spores, a macro- and a micro-conidium of *Fusarium culmorum* (*b*) a dry conidium of *Helminthosporium sativum* (perfect state is *Cochliobolus sativus*). These two species, causing foot-rots of cereal crops, will be discussed in Chapters 7 and 8. There are also a great many fungi, particularly in the Oomycetes and some other classes, in which the asexual propagules are *zoosporangia*, germinating to produce *zoospores*, which are propelled by the rapid movement of flagella. Such fungi are typical of aquatic habitats but are also active in wet soils, where some of them cause widespread diseases of both wild and cultivated plants. Large areas of native eucalypt forest in Australia have been devastated by *Phytophthora cinnamomi* (Oomycetes), largely because the natural water-holding and drainage system of the soil has been ruined by ecologically reprehensible procedures of timber extraction; resulting mass movement of water, and even surface flooding, has much favoured multiplication and spread by this once unnoticed pathogen.

In the examination of soil, either microscopically or by culturing of the fungi (Ch. 6), imperfect states of fungi are seen more commonly than the perfect states. A useful guide for identification has been provided by Barnett and Hunter (3rd. ed., 1972) in their *Illustrated Genera of Imperfect Fungi*. Information about genera and their classification, together with much else needed by practising mycologists, is given by G. C. Ainsworth (6th. ed., 1971) in his *Ainsworth & Bisby's Dictionary of the Fungi*. A comprehensive and vivid account of spore-dispersal mechanisms in fungi is given by C. T. Ingold (1971) in his *Fungus Spores*.

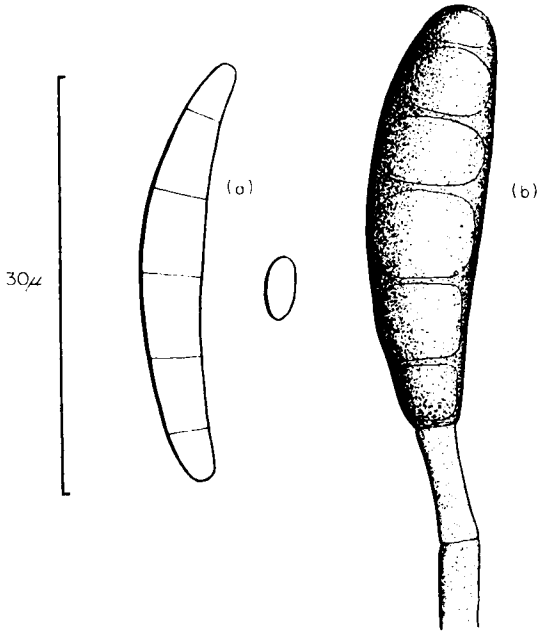

FIG. 5. Asexual spores of *a. Fusarium culmorum*; *b. Helminthosporium sativum.*

MORPHOGENESIS OF FUNGAL ORGANS

Many of the septate fungi show the characteristic of *associated hyphal growth*, whereby fungal tissues can be built up. In the production of such tissues, two or more types of hyphae may be differentiated; interlocking of hyphae is effected both by interweaving growth and by anastomoses between adjacent hyphal tips. Synthesis of different kinds of fungal tissue culminates in the fruit bodies of the higher or *macro-fungi*, e.g. the saucers, cups and stalked heads produced by ascomycetes and the toadstools, fungal brackets on trees and puff-balls produced by basidiomycetes. Such fruit bodies show a wide range of flesh texture from fleshy through cartilaginous to leathery or woody. This range attests the remarkable versatility of the fungal hypha as a unit of construction; tissues and organs comparable with those of higher plants can be built up by a mode of construction that is radically different.

Mycelial strands

Amongst fungi that produce these organs of translocation, mycelial strands can be built up in several different ways. A single example will suffice here: the strand formed by *Serpula lacrimans* (formerly known as *Merulius lacrymans*), a fungus causing the notorious dry rot of house timber. These strands were studied in remarkable detail by the German mycologist, R. Falck (1912); his observations were later confirmed and amplified by Gillian M. Butler (1957, 1958). Strands are typically formed as *S. lacrimans* traverses a surface devoid of nutrients, such as plaster, brickwork or concrete, in the humid atmosphere of a poorly ventilated house and especially in that just above the foundations. Such a surface provides no nutritents for the fungus, which on a wood substrate obtains sugars by the hydrolysis of cellulose. Strands develop around one or more leading hyphae growing parallel, out from a wood food-base over an inhospitable surface in a humid atmosphere; these leading hyphae become closely and compactly enwrapped by their own slender branches, which Falck (1912) aptly dubbed "tendril hyphae". Initiation of a strand begins some way behind the apices of the leading hyphae and gradually develops forwards. In the mature strand, the original leading hypha(e) may be further differentiated by development of wall thickening and by dissolution of septa so as to give a more or less continuous tube, as observed by Falck (1912). Thick-walled, fibrous hyphae may also be differentiated within the developing strand. The first stages in strand construction are shown in Fig. 6.

Why do strands thus develop when *S. lacrimans* traverses a nutrient-free surface? Teleological speculation has suggested some reasons: (1) economy of nutrients, insofar as the tendril hyphae are limited in their growth (2) protection of the main conducting hyphae against desiccation, and also against the hydrostatic pressure developing during translocation of nutrients from the food-base, as D. H. Jennings (1981) has suggested (3) aggregation of hyphae into a strand secures the pooling of hyphal energy of growth, thus providing the maximum possible concentration of cellulase enzymes for initiation of wood decomposition when a substrate is encountered. Stranding is thus said to increase the *inoculum potential* of the fungus; this concept was developed by Garrett (1956) to describe the invasive force needed by a pathogenic root-infecting fungus for the over-

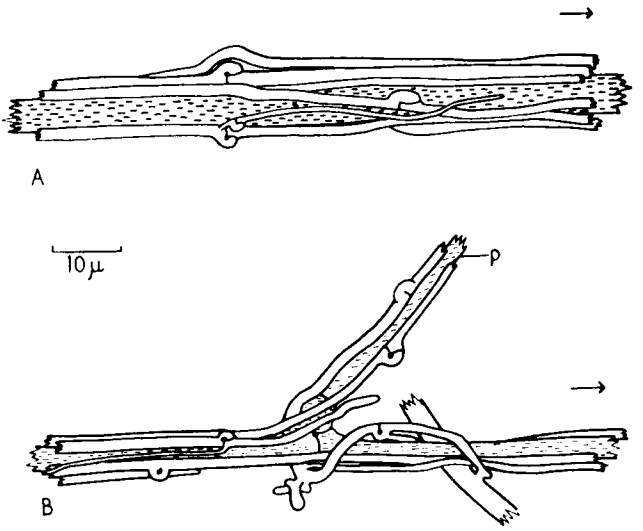

FIG. 6. Details of mycelial strand construction in *Merulius lacrymans*. *A*, wide main hypha with a covering of narrower tendril hyphae; *B*, tendril hyphae growing along a wide main hypha and its free primary branch (*p*). (After G. M. Butler (1958). By permission, *Annals of Botany*.)

coming of host-plant resistance to invasion; for later discussions see Garrett (1970, pp. 9–13) and R. Baker (1978).

Teleological speculation, as above, asks the question "why?". For many research workers, this is an essential aid to thinking and therefore to further research but the questions can rarely be answered with finality. If we ask the question "how?", then we are seeking an answer in terms of the actual mechanism of strand formation. Sarah C. Watkinson (1971*a*, *b*) has indeed provided a satisfactory explanation of *how* the strands of *S. lacrimans* develop. She has shown in various ways, including use of radio-active tracer phosphate, that the leading hyphae of *S. lacrimans* exude nutrients when they are translocating, much as a young, active root produces nutrient exudates. The tendril branch hyphae respond to this nutrient stimulus by growing closely around their parent hypha. This explanation is compatible with all the observations about strand development, including their late appearance on culture media, when soluble nutrients have been

reduced to a level at which they no longer mask the nutrient stimulus provided by exudation from the main hyphae.

Rhizomorphs

In contrast with the gradual building-up of a mycelial strand, a typical rhizomorph grows fully fashioned from an apical meristem, like that of a root. Rhizomorphs are produced by the fungus *Armillaria mellea*, which invades the roots and collar of forest and plantation trees over most of the world; it is known as the "honey fungus" from the tawny colour of its toadstools and as the "bootlace fungus" from its mature black rhizomorphs. The apical region of such a rhizomorph is shown in Fig. 7.

Activity of the apical meristem, situated *ca.* 25μm from the extreme tip, has been described by J. J. Motta (1969, 1971); forwards of itself, the meristem produces cells that form a mucilage-coated structure like a root cap. Behind and to the sides, meristematic cells produce the loose tissue of the central medulla, flanked by a more compact cortex, outside of which lie the narrower, thicker-walled and eventually melanized cells of the rind. Later in development, the loose tissue of the medulla collapses, leaving a central air-channel extending from the food-base of the rhizomorph to its apical region. This air canal supplies oxygen from the food-base, which is in an aerobic situation, to the apical region and meristem. This enables the rhizomorphs to grow straight down through a vertical column of nutrient agar, which is almost anaerobic; other fungi can grow as mycelium to only a few millimetres below the agar surface.

The rhizomorph of *A. mellea* still fascinates mycologists by its unsolved mysteries. How does an apical meristem become organized within a colony of unorganized mycelium? If we take a disk (4 mm diam.) from the margin of a purely mycelial colony on agar and lay it on a fresh plate of nutrient agar, a ring of white rhizomorph initials regularly appears around the margin of the original inoculum disk after 7 days at 25°C (Plate 1, Fig. 1). The young rhizomorphs, which usually grow at 5 or 6x the rate of the mycelial margin of the colony, quickly grow out beyond it; the result of this is a deeply lobed colony, with the fringing mycelium from the rhizomorphs producing the lobes (Plate 1, Fig. 2). In one isolate of *A. mellea* studied by J. Rishbeth (1968), rhizomorphs grew 13x as fast as a colony of unorganized mycelium. The reason for this has not yet been explained, but in

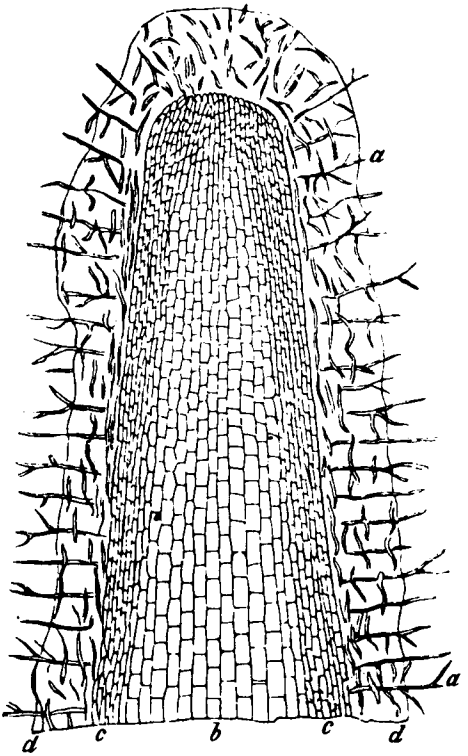

FIG. 7. Apex of rhizomorph of *Armillaria mellea* in longitudinal section. *a*, mantle of filamentous hyphae; *b*, central conducting cells; *c*, cells of the rind; *d*, boundary of enveloping mucilaginous layer (After R. Hartig).

a recent review of fungal morphogenesis Watkinson (1979) has suggested that rate and magnitude of cell extension behind the meristem may be as important as rate of cell division in maintaining this high growth rate of rhizomorphs.

FRUIT BODIES OF THE HIGHER FUNGI

Some ten thousand species of macro-fungi have been described and named; their fruit bodies exhibit an extremely wide range in size, shape and

construction, but all of them serve the same function, i.e. production and dispersal of the spores resulting from the process of sexual reproduction (see Ch. 5).

Spore dispersal from a fruit body

As an example, we can consider the most widely known fruit body, that of the common field mushroom (*Agaricus campestris*). The fruit body, illustrated diagrammatically in Fig. 8, consists firstly of a stalk (the *stipe*), which is connected by white mycelial strands with the substrate of the fungus within the soil. Surmounting the stipe is the cap (*pileus*). In the young fruit body, or "button", the underside of the pileus is completely enclosed by a membrane of white tissue, the *partial veil*. As the pileus expands, the partial veil is ruptured, and the remains of it persist as a ring of tissue (the *annulus*) attached to the upper part of the stipe. From the underside of the expanded pileus, a series of radially arranged, plate-like gills (pink in colour) projects vertically downwards.

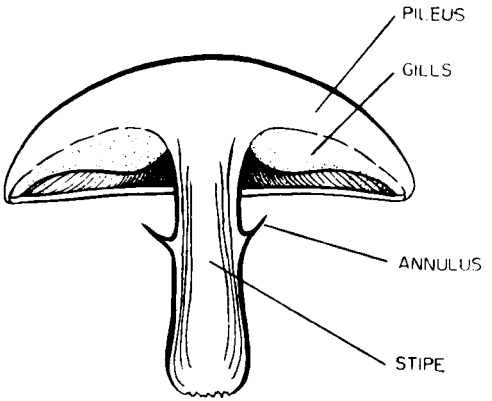

FIG. 8. Diagrammatic section of the common mushroom.

This is all that can be seen with the naked eye, or with a hand lens. But a thin section, cut across one of the gills with a razor and viewed under the microscope, will show that both surfaces of the gill are clothed with a spore-bearing layer, the *hymenium*. The hymenium is made up of swollen cells

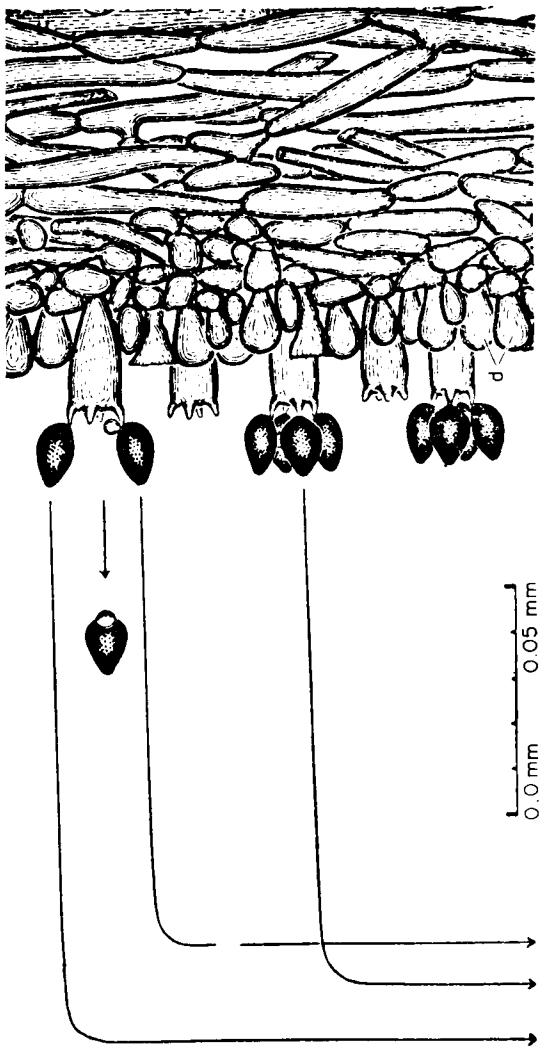

FIG. 9. Basidiospore formation and discharge from one of the gills in a fruit body of *Panaeolus campanulatus* (After A. H. R. Buller, *Researches on Fungi, vol. II.* By permission, Longmans, Green and Co.).

known as *basidia*. At the distal or outwards-facing end of each basidium, four slender projections (the *sterigmata*) arise; on each sterigma is ultimately produced a single *basidiospore* (Fig. 9). When mature, the four basidiospores are rapidly discharged in succession from the four sterigmata, to a horizontal distance of about 0.1 mm. This distance is sufficient for the spore to be carried clear of the hymenium layer on which it was produced, but not far enough for it to hit the hymenium on the adjacent gill opposite. In consequence of this arrangement, the discharged basidiospores drop safely down in the space between adjacent gills. The efficiency of the arrangement, however, obviously depends upon an accurate vertical alignment of the gills. This is achieved by two methods of adjustment. The stipe is negatively geotropic, i.e. it grows against the direction of the force of gravity and thus vertically upwards. This ensures that the pileus, which grows diageotropically (i.e. transversely to the direction of the geotropic pull), will be aligned horizontally. The gills themselves are positively geotropic, and in their downwards growth make a final and accurate alignment with the vertical.

In addition, however, to securing an approximately horizontal alignment for the pileus, the stipe fulfils another function, by raising the whole pileus to a sufficient distance from the ground. It was formerly supposed by mycologists that the distance travelled by a fungal spore dropped from a fruit body was determined by a simple parallelogram of forces, the vertical component being fall under the influence of gravity and the horizontal component being provided by the wind. Some naturalists may indeed have wondered why the average length of stipe in large species of toadstool is often short in relation to size of the pileus; stipes much longer than the average are mechanically possible, as shown by the tall parasol mushroom (*Lepiota procera*), which typically grows amongst tall grass. The answer to this query was not provided until some 60 years ago, and came from a fundamental study of the movement of air-borne particles in the atmosphere. It was shown that, except for a relatively thin layer of air (the *boundary layer*) bordering the ground and other surfaces, which is in laminar flow, the atmosphere is in a state of turbulence (except during windless periods). This turbulence is generated both mechanically (i.e. by the action of wind) and thermally. For small bodies of the size of fungal spores, pollen grains and smoke particles suspended in turbulent air, the downwards force of gravity is largely counteracted by atmospheric

turbulence; a fungal spore has an approximately equal chance of being carried upwards or downwards by the eddies making up the turbulence, though a volume of heated air moving upwards will produce an upwards bias in spore movement. It follows from this explanation that the stipe of a toadstool must be of such a length that it raises the pileus to a height at which spores can be dropped into the turbulent air; a height appreciably in excess of this will bring a return, in terms of increased efficiency in spore dispersal, incommensurate with the expenditure of extra fungal material upon the stipe. Factors determining the movement of fungal spores and other living particles in suspension in the atmosphere have received a through and fundamental treatment from P. H. Gregory (2nd ed., 1973) in his book *Microbiology of the Atmosphere*.

Morphogenesis of fruit bodies

Whereas the external forms of toadstools, brackets and other types of fruit body are well known in all their variability to mycologists, the same cannot be said of their morphogenesis. The problems in morphogenesis presented by these complex organs are amongst the most formidable in all mycology. Some 50 years ago, E. J. H. Corner (1932*a*, *b*) initiated an entirely new approach to this subject, by the method that he has named *hyphal analysis*. Corner has distinguished three principal types of hyphae that take part in the construction of fungal fruit bodies:

(1) *Generative hyphae*. These are thin-walled, branched, usually septate, with or without clamp connexions. They give rise to other types of hyphae and to the hymenium.

(2) *Skeletal hyphae*. These are thick-walled, branched or unbranched, commonly aseptate, straight or slightly flexuous.

(3) *Binding hyphae*. These are thick-walled, much branched, aseptate, interwoven and often coralloid. They bind the skeletal and generative hyphae together.

Corner has given the name of *monomitic* to those fruit bodies with one system of hyphae (generative); the name *dimitic* is applied to fruit bodies with two hyphal systems (generative + skeletal), while *trimitic* fruit bodies possess all three hyphal systems (generative + skeletal + binding). Fleshy fruit bodies are usually monomitic; hard and tough fruit bodies are more commonly dimitic or trimitic.

The use of hyphal analysis is displayed in Corner's (1950) *A Monograph of Clavaria and Allied Genera.* The technique of hyphal analysis is emphatically not one for the dilettante; the aims of the method have been epitomized by Corner in the following sentence from his preface to the *Clavaria* monograph: "Not until the hyphae have been traced through their elaborate organization in fruit bodies from the mycelium to the spores which are finally abstricted from their tips can the true problem of the higher fungi be seen." It has been claimed (and with substantial evidence in support of the claim) by Corner and his followers (e.g. Cunningham, 1947, 1954) that the hyphal construction of the fruit body is a more fundamental and reliable guide to the taxonomy of a species than is the gross morphology (see also a review by Teixeira, 1962).

CHAPTER 5

GENETICS AND CLASSIFICATION OF FUNGI

Some form of sexual reproduction, if this term be used in its most inclusive sense, is found in every group of organisms. Different individuals of any species carry a different assortment of hereditary potentialities; this statement must be qualified, however, by saying that plants and some animals can propagate themselves (or be propagated by man) by purely vegetative means, i.e. through asexual reproduction. All the individuals thus propagated will have an identical hereditary constitution, and are said to constitute a *clone*. Sexual reproduction between two unlike individuals of a species results in a shuffling or reassortment of the hereditary determinants, or *genes*; this takes place entirely at random and results in progeny (the "F_1 generation") that differ in genetical constitution both amongst themselves and from either of their parents. Regularly recurring sexual reproduction thus provides a pool of genetical variation amongst the population of any species, and this is the raw material upon which natural selection works. When Charles Darwin published in 1859 *The Origin of Species by means of Natural Selection*, there remained 7 years to go before the clue to the origin of variations in a population was published by Gregor Mendel in the Transactions of the Brünn Natural History Society in 1866. Darwin remained unaware of Mendel's paper up to the time of his death in 1882, and so did the scientific world in general up to the end of the century, when Mendel's results were independently confirmed by C. Correns, E. Tschermak and H. de Vries, and his paper was unearthed. Both Darwin and his supporters had been well aware that the provision of an ample supply of heritable variation within a population was essential for the working of natural selection as he had postulated, and ignorance of the

way in which this was, or might be, provided was the chief stumbling block to general scientific acceptance of the Darwin–Wallace hypothesis. The development of modern genetics, and particularly the work of R. A. Fisher (1930) and other neo-Darwinians, has now largely removed this difficulty.

The hereditary material of any eukaryotic organism is carried within the nuclei of its cells, and can be seen under the microscope at the time of nuclear division (after a suitable staining procedure) in the form of linear bodies known as *chromosomes*. The number, size and shape of the set of chromosomes is characteristic for the species. The expression of hereditary characters in any individual is determined by a large number of *genes*, which are arranged in linear order along the several chromosomes. The order in which the genes lie along their particular chromosomes has been worked out for a number of species by mathematical calculation from the results of breeding experiments, so that *chromosome maps* have been constructed showing positions of various genes. The expression of any particular hereditary character may be determined by the action of more than one gene; the effect of these genes on the development of the individual is influenced to some extent by the whole gene complex, and also by the action of the environment. During the last 30 years, great advances have been made in determining the chemical nature of the genes and their probable mode of action in causing the cell to carry out particular syntheses. Nevertheless, the above account will suffice for our present purpose of outlining the nature of genetical mechanisms in fungi, though some reference to these advances has been made earlier, in the section on the eukaryotic cell (Ch. 3).

NUCLEAR DIVISION

During the division of a vegetative cell in the body of any organism, the nucleus also divides; at this time, there is then a longitudinal splitting of each chromosome, with the result that an identical set of daughter chromosomes passes to each of the two daughter cells. During the act of sexual reproduction, there is a fusion between male and female *gametes* and a subsequent fusion between male and female nuclei. The nucleus resulting from this fusion thus has two complete chromosome sets, one derived from the male and the other from the female parent, and is said to be *diploid*. This contrasts with the single set of chromosomes contained in the nuclei of the

male and female gametes, which are said to be *haploid*. It is obvious that somewhere in the life cycle of the organism, a compensatory reduction in the number of chromosomes back to the haploid number must occur. This reduction is effected by means of a special type of nuclear division, known as a *reduction division* or *meiotic division*; the normal nuclear division of vegetative cells is known as *mitosis*. In higher plants and animals, meiosis takes place during formation of the gametes, which alone are haploid; the new individual becomes diploid as a result of nuclear fusion after the union of sperm and egg cells, and remains diploid for the rest of its life. In most of the fungi, on the other hand, meiosis most commonly takes place not just before, but soon *after*, fusion of male and female nuclei. Such a fungal individual is therefore haploid throughout its life; this is considered by geneticists to be a condition inferior to that of the diploid state, which allows more latitude for both the alternatives of reinforcement or suppression of the effects of any particular gene. This permits a greater flexibility in the development of the organism, according to the demands of the environment.

In a meiotic division, halving of the number of chromosomes is effected as follows. It is necessary first to remember that, in a mitotic division, identical sets of daughter chromosomes (derived by longitudinal splitting of the original chromosomes) go to each of the two daughter nuclei. In a meiotic division, on the other hand, one daughter nucleus receives one whole chromosome, which for simplicity of explanation we will label "male-parent-derived"; the other daughter nucleus receives the other whole chromosome of the homologous pair, which we can label "female-parent-derived". The number of chromosomes is thus halved, back to the haploid number, but an equally important consequence of meiosis is that the resulting haploid nuclei have different genetical constitutions, according to the proportion of "male-parent-derived" and "female-parent-derived" chromosomes that each contains. If, for example, there are four homologous pairs of chromosomes in the original diploid nucleus, then there are sixteen possible ways in which the four pairs can be split between the two haploid daughter nuclei resulting from meiosis.

Thus the reassortment of whole chromosomes provides for a wide range of variation in the genetical constitution of the products of nuclear meiosis. It does not, however, provide for a reassortment (amongst possible progeny of the original sexual pairing) within the group of genes that is

located on a single chromosome; these genes are therefore said to be *linked* in inheritance. This linkage, however, is frequently broken during the course of meiosis, as a result of what might, at first sight, appear to be a mechanical imperfection in the process. It is necessary first to explain that, either just before or early in the meiotic division (or what is more properly called "the first division of meiosis"), the paternal and maternal chromosomes each become duplicated to give the two linear halves (called *chromatids*) that become daughter chromosomes in a mitotic division. At the same time, paternal and maternal chromosomes (each partially separated into two identical chromatids) become closely paired with one another. The results of genetical analysis of breeding experiments have shown that, during this very close association between the paternal and maternal pairs of chromatids, either of the maternal chromatids may exchange one or more linear segments with either of the paternal chromatids. Thus although paternal and maternal pairs of chromatids separate as complete chromosomes at the end of the meiotic division, the two pairs of chromatids will no longer be either wholly "paternal" or wholly "maternal", because crossing-over will have occurred and segments have been exchanged. *It therefore happens that the two chromatids in each pair will be unalike*, i.e. all four chromatids in the two pairs will be different. The first mitotic division after the meiotic one will therefore produce daughter nuclei that are unalike, and not identical as in the case of a normal mitotic division. Owing to this behaviour of the chromatids in crossing-over, the reassortment of genes is thus not complete until a mitotic division has followed the meiotic one. Meiosis is therefore said to consist of two nuclear divisions: a meiotic one followed by a mitotic one, but the latter is not a normal mitosis because it results in the production of unlike daughter nuclei. We see, therefore, that as a result of crossing-over in meiosis, almost any type of reassortment of genes as a final outcome of sexual reproduction is possible, even though the mathematical probability of occurrence of any one particular type of reassortment may be extremely low.

GENETIC MECHANISMS IN THE FUNGI

The foregoing brief account of nuclear division has been a necessary prelude to consideration of genetic mechanisms in the Fungi, which as a group show some peculiar features of much interest to geneticists. Those

readers who wish for a fuller introduction to general genetics cannot do better than read H. L. K. Whitehouse's (1965) *Towards an Understanding of the Mechanism of Heredity*, in which a difficult subject is explained with remarkable clarity and precision. The difficulties are not confined to the beginner; many biologists not directly concerned with genetics must find, as I have certainly done, that it is easy to forget even the basic phenomena of genetics and that one has frequent need to refresh one's memory. Mycologists who are not professional geneticists are fortunate in being able to find an unusually lucid account (which any biologist can understand) of fungal genetics in a series of theoretical papers by Whitehouse (1949*a*, *b*, 1951). These also are strongly recommended for further reading.

Table 4 provides an outline classification of the mycelial division (Eumycota) of the Fungi into its five subdivisions: Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina, and Deuteromycotina. These were briefly mentioned in Chapter 4, and the three classes of the sub-division Deuteromycotina (Fungi Imperfecti) were set out in Table 3. In Table 4, classes within each sub-division have been named only when necessary for elucidation of the text. The principal changes (other than taxonomic ones) that have had to be made in Table 4, when compared with the corresponding Table 1 in the first edition (1963) of this book, have been concerned with the class Oomycetes; the mycelia of these fungi were formerly thought to be haploid, with meiosis occurring during the first divisions of the oospore nucleus. The correction of this belief was largely due to a paper by Eva Sansome (1963) on the cytology of *Pythium debaryanum*, a widespread soil pathogen causing "damping off" and similar diseases of seedlings and young plants. She showed that meiosis occurs during gametogenesis, i.e. the formation of male (*antheridia*) and female (*oogonia*) sexual organs. The product of their sexual fusion is therefore diploid, and the diploid condition is maintained throughout the life of the mycelium derived from the oospore, until haploid gametangia are produced. The results of much further work, both cytological and genetical, by Sansome and others have shown that both oospores and mycelium are diploid in various other genera of the Peronosporales (an important order of the Oomycetes, containing many plant pathogens): *Phytophthora*, *Peronospora*, *Sclerospora*, *Bremia* and *Albugo*. Comprehensive reviews of the work establishing this general conclusion have been provided by Brasier and Sansome (1975) and by Caten and Day (1977).

TABLE 4. CLASSIFICATION AND GENETIC MECHANISMS OF FUNGI IN THE DIVISION EUMYCOTA

Taxonomic sub-division	Taxonomic class	Character of mycelium	Mechanism whereby fusion of parental nuclei is effected	Mechanism providing for outbreeding (in at least some species of each group)	Spore type produced after genetical recombination
Mastigomycotina	Oomycetes	Aseptate, diploid	Sexual organs, morphologically dissimilar	Two-allelomorph hetero-thallism, at one chromosome locus (bipolar)	Diploid oospores
Zygomycotina	Zygomycetes	Aseptate, haploid	Sexual organs, morphologically alike (except sometimes in size)	Two-allelomorph heterothallism, at one chromosome locus (bipolar)	Haploid sporangiospores
Ascomycotina		Septate, haploid	Morphological differentiation and functioning of sexual organs in only a minority of species	Two-allelomorph heterothallism, at one chromosome locus (bipolar)	Haploid ascospores (typically 8) in an ascus
Basidiomycotina	Hymenomycetes and Gasteromycetes	Septate, dikaryotic (paired haploid nuclei)	Anastomoses between vegetative mycelia, or between mycelia and oidia	Multiple-allelomorph heterothallism at one locus (bipolar) or at two loci (tetrapolar)	Haploid basidiospores (typically 4) on a basidium
Deuteromycotina (Fungi Imperfecti)		Septate, haploid	Parasexual cycle		Haploid asexual spores

Secondly, the occurrence of two mating types (*bipolar heterothallism*, see 5th column, Table 4, and section on heterothallism) has been established for various species in the genera *Phytophthora*, *Pythium* and *Bremia*, though homothallic isolates of these fungi also occur. These two discoveries, of mycelial diploidy and of bipolar heterothallism, have led to a fundamental revision of the former view that the Oomycetes could justifiably be called "Lower Fungi". As noted above, the diploid mycelial state permits more scope for either reinforcement or suppression of the effects of any particular gene within the genome, as selection pressures may dictate. Secondly, the probably widespread occurrence of bipolar heterothallism within the Oomycetes provides a mechanism for outbreeding, and hence for a broader spectrum of genetic recombination, as efficient as that found in the Ascomycotina.

SEXUAL FUSION BETWEEN FUNGI

Our first example of sexual behaviour will be that in the Oomycetes. In these fungi, the male organ (*antheridium*) applies itself by growth closely to the female organ (*oogonium*); the intervening cell walls are dissolved, the contents of the antheridium pass into the oogonium, and fusion between male and female nuclei follows to produce a diploid fusion nucleus. The product of fertilization (or *zygote*) develops into a thick-walled *oospore*, which may require a longish period for ripening, especially in some species of *Phytophthora*, before it is ready to germinate. According to environmental conditions, the product of germination may be either diploid zoospores or a diploid mycelium.

Some species of Oomycetes are *dioecious*, which means that, as in many flowering plants, male and female organs are borne on separate individuals. This is one way, though by no means the only way, in which *outbreeding* can be assured, and with it a supply of different genes. To speak of "different genes" is not strictly correct, however, and a word of explanation becomes necessary on this point. If we consider a particular gene, A, which is related to the expression of some particular character, then some form of this gene is *present at a particular chromosome locus in all individuals of the species*. This gene A, however, exists throughout the population of individual members of the species in a number of different forms or *alleles*, which we can designate as A_1, A_2, A_3, etc. These different alleles vary in the extent to

which they can affect the expression of the particular character in the individual possessing them; the specific genetical effect of any particular allele will also be affected by the rest of the gene complex belonging to the individual, and by the environment in which it develops. Outbreeding, therefore, does not bring in different genes; what it does bring in are different *alleles*. Some genes are known to exist in as many as 100 different allelic forms. Such allelic forms arise as a result of what is called a *mutation*; this is a spontaneous (or "accidental") alteration in a chromosome affecting one or more genes. It is the accumulation of mutations that has provided a pool of variation within the populations of all species of organisms.

Whereas dioecism in some oomycete fungi brings with it the great genetical advantage of outbreeding, it is now necessary to record the puzzling fact that the majority of oomycete species, in some groups at least, are *monoecious*, i.e. oogonia and antheridia are borne on the same mycelium, so that no outbreeding can occur. The most likely explanation at present available for the widespread occurrence of monoecism in the Oomycetes is to suppose that it carries the same advantages as cleistogamy (the production of flowers that never open, so that only self-pollination can occur) in some species of flowering plant. These advantages are (1) greater certainty of pollination and consequent setting of seed (2) a degree of genetical stability that may be advantageous in a stable environment in which the species occupies a secure ecological niche.

So in the Oomycetes, we see that fusion between male and female nuclei is effected through the agency of morphologically differentiated sexual organs, and that the male and female organs are distinct in appearance. In the class Zygomycetes, union occurs between less obviously differentiated fertile branches of the mycelium, known as *gametangia*; the product of fusion is a thick-walled *zygospore*. Male and female gametangia are alike in form, though in some species the assumed "female" gametangium is consistently larger than the "male" one. In the Ascomycotina, the female or receptive organ may or may not be morphologically distinguishable; it fuses with a male hypha (or with an *oidium*—a spore carrying a single haploid nucleus—derived from a male hypha) which is difficult to distinguish (except by function) from a vegetative hypha. Finally, in the Hymenomycetes (toadstool and bracket fungi) and Gasteromycetes (puffball, etc fungi) of the Basidiomycotina, no sexual organs are produced; the

haploid mycelia arising from single haploid basidispores fuse with one another by *vegetative anastomosis*, or a haploid mycelium may be "diploidized" by fusion with one or more oidia from another mycelium. In the higher Basidiomycotina, therefore, the mycelium as a whole has taken over the function of the specialized sex organs that are still found in the Oomycetes. The two unlike types of nuclei present in a diploidized mycelium do not fuse at this stage, but they associate themselves in the form of unlike pairs; such a pair of genetically unlike nuclei associated together in growth and division is known as a *dikaryon*. It is possible that the dikaryotic condition of the mycelium that is found in the Basidiomycotina may carry with it some of the advantages of the diploid nuclear condition.

We can thus trace a progressive reduction in the role of morphologically differentiated sexual organs as the site of nuclear fusion as we pass from the Oomycetes through the Ascomycotina up to the higher Basidiomycotina. In the Oomycetes and Zygomycetes, anastomoses between mycelia do not seem to occur, and fusions are restricted to sexual organs; male and female organs are morphologically distinct in the Oomycetes, but usually not in the Zygomycetes. In some species of the Ascomycotina, recognizable sexual organs are functional; in others, sexual fusions take place between vegetative mycelia. In the higher Basidiomycotina, the function of sexual organs in providing for the association and eventual fusion of parental nuclei has been taken over by the vegetative mycelium as a whole.

In contradistinction to this reduction in the importance of sexual organs as we pass from the Oomycetes to the higher Basidiomycotina, we find a progressive increase in size and complexity of the fruiting structures on or in which the spores resulting from the sexual process are borne. The oospores of the Oomycetes and the zygospores of the Zygomycetes are borne naked on the mycelium (except in the family Endogonaceae, in which the zygospores are enmeshed in a fruiting body of loosely woven mycelium). Amongst the Ascomycotina, the asci are borne within or upon fruiting bodies produced by associated hyphal growth; in complexity of organization, these fruiting bodies culminate in the fruiting cups of the Discomycetes. The maximum both in complexity of organization and in size is reached in the higher Basidiomycotina. The basidia are variously produced on gills or spines, or lining the inside of pores or tubes, on the underside of toadstools growing out of the ground, or of fungus brackets growing out of standing trees or of fallen branches. Basidia are also

produced within puff-balls and in other but less common ways too numerous to mention.

HETEROTHALLISM AS AN OUTBREEDING MECHANISM

We can now pass on to consider fungal mechanisms that provide for outbreeding (Table 4, 5th column). One such mechanism—dioecism in the Oomycetes—has already been discussed, together with the advantages accruing from outbreeding. A much more efficient and widespread mechanism that secures outbreeding in the Fungi, however, is *heterothallism*. Before explaining heterothallism, it is essential to distinguish between this phenomenon and that of sex, because some mycologists—and certainly I myself—have experienced great initial difficulty in getting the distinction clear. Sexual mating, which in fungi may be effected in a variety of ways as we have just seen, provides for fusion between unlike parental nuclei, and therefore for a genetical reassortment of characters in the eventual progeny of the mating. Heterothallism promotes the occurrence of outbreeding, and therefore subserves the same end as the sexual process, which it renders more efficient. Heterothallism is not the same as sex; it is a refinement superimposed upon it.

The discovery of heterothallism in its simplest form amongst species of the order Mucorales (Zygomycetes) was made by the American mycologist A. F. Blakeslee (1904). He found that some species were heterothallic, and that zygospores were produced only by fusion between gametangia belonging to separate, and *unlike*, mycelia. For any heterothallic species, strains could be classified into one or other of two types, designated (+) and (−), respectively; sexual fusion occurred only between strains of unlike, or "compatible", mating type. Not all species of the Mucorales were found to be heterothallic; some were *homothallic*, i.e. all mycelia were alike in mating type, and sexual fusions regularly occurred between gametangia on the same mycelium. The simplest type of heterothallism thus discovered by Blakeslee is now attributed to the occurrence of a gene at the so-called "*incompatibility locus*"; this gene exists in the form of two alleles, for (+) and (−) mating types, respectively. As there are only two alleles at one chromosome locus, the phenomenon was originally termed bipolar heterothallism. Later on, H. Kniep (1920, 1922) discovered that in the hymenomycete species, *Schizophyllum commune*, there was not one but two

incompatibility loci; this type of heterothallism has accordingly been named *tetrapolar heterothallism*. It has since been found that tetrapolar heterothallism, as well as bipolar heterothallism, is widespread amongst species of the class Hymenomycetes (Basidiomycotina).

The advantages of the more complex types of heterothallism common amongst the Hymenomycetes can be simply explained. Two-allelomorph bipolar heterothallism, at one chromosome locus, results in the occurrence of (+) and (−) strains, and makes outbreeding a complete certainty. But this certainty is achieved at the expense of 50% of "sexually unprofitable encounters", because the chances that a (+) strain will encounter a (−) strain (and not another (+) strain) are 1 in 2. A reduction in the percentage of unprofitable encounters to a negligible proportion has been brought about through the evolution of *multiple-allelomorph heterothallism* in the Hymenomycetes and Gasteromycetes. Here, at one incompatibility locus, are not two alleles but a much larger number, and quite commonly about 100. If there are 100 alleles, then the percentage of sexually unprofitable encounters is reduced to 1%. A multiple-allelomorph series can also occur at each of two distinct chromosome loci (tetrapolar); these loci are probably on two different chromosomes, or else so far apart on one chromosome that segregation occurs freely by crossing-over at meiosis. It will suffice to say here that the tetrapolar arrangement actually increases slightly (theoretically to 1.99%) the proportion of sexually unprofitable encounters, but it reduces the chances of inbreeding between mycelia derived from basidiospores produced on the same fruit body. For multiple-allelomorph heterothallism at one locus, there is a 50% chance of successful mating between mycelia originating from basidiospores of the same fruit body; for multiple-allelomorph heterothallism at two loci, this chance has been reduced from 50 to 25%.

THE FUNGI IMPERFECTI

There finally remains for consideration the sub-division Deuteromycotina (Fungi Imperfecti). As many of these imperfect fungi are widespread and successful species, the apparent absence of any mechanism for sexual recombination in their life-cycles once appeared to cast a grave doubt upon the claims made for the value of some kind of sexuality. This doubt has been dispelled by a number of discoveries. Firstly, it was found that

anastomoses between vegetative mycelia, and particularly between young mycelia, are much more frequent than was formerly supposed. Mycelia containing unlike nuclei arise from such anastomoses. General attention was first drawn to this by Hansen and Smith (1932), who demonstrated that when "mycelial" and "conidial" strains of *Botrytis cinerea* were allowed to anastomose together in culture, a series of single-spore cultures from the fusion mycelium produced colonies showing various gradations in intensity of sporulation between the sparsely sporing "mycelial" parent and the heavily sporing "conidial" parent. This was subsequently named the *dual phenomenon* by Hansen (1938), but is now referred to as the condition of *heterokaryosis*, i.e. the association of genetically unlike nuclei within a single mycelium. The characters of the mycelium can thus be determined, to some extent at least, by the relative proportions of different nuclei in such an association, which may be relatively stable in a constant environment. But if the environment changes, and particularly if the composition of the substrate changes, then rates of multiplication of different kinds of nuclei may be differently altered, so that the composition of the heterokaryon is changed. Heterokaryosis thus provides a mechanism of variation in a composite mycelium, whereby the characters of the organism can change in response to a change in environment or substrate. This mechanism appears to be peculiar to the Fungi.

Analysis of heterokaryosis led Pontecorvo, Roper and Forbes (1953) to the important discovery that, in the heterokaryotic mycelium of an imperfect fungus, occasional fusions occurred between two unlike haploid nuclei, and that this led eventually to new combinations of characters. This phenomenon has been named the *parasexual cycle* (Pontecorvo, 1956). Subsequent work on the parasexual cycle suggests that the name of "imperfect fungus" will eventually come to appear inappropriate in reference to genetical efficiency, which is the ultimate justification of all forms of sexual, or parasexual, reproduction.

CHAPTER 6

STUDYING THE SOIL FUNGUS FLORA

The first isolation of soil fungi in culture was made by I. Adametz (1886) in Germany and he was followed by Oudemans and Koning (1902) in the Netherlands and then by others in the early years of the present century. The real importance of fungi in the soil economy was not fully established until some 50 years ago and another 20 years were to elapse before the ecology of soil fungi became as popular a study as it is today. The early years of any branch of plant ecology must be occupied chiefly by a study of the whole flora comprising the community occupying any habitat under investigation; this is called a *synecological study*. It is later followed by a more intensive *autecological study* of particular species that may be of special scientific interest or of great economic importance for applied biologists. So soil mycologists first had to collect information about the genera (and species) of at least the commoner fungi in the soils that they were studying. So the compilation and comparison of fungal *floristic lists* was a first task for soil mycologists all over the world. As time went on, these synecological studies were increasingly supplemented by autecological studies of economically important soil fungi. As noted in Chapter 1, such fungi are chiefly the mycorrhizal fungi, making a positive contribution to crop growth and yield, and the pathogenic root-infecting fungi, making a negative one. Work with these fungi will provide most of the examples to be discussed in following chapters. The present chapter will now describe the more general studies.

STUDIES OF FUNGAL COMMUNITIES BY DIRECT MICROSCOPICAL OBSERVATION

The first and most fundamental problem of soil mycology was posed by the assertion of some bacteriologists (e.g. Conn, 1917) that fungi do not live

in the soil or take any active part in the decomposition of organic matter therein, but are present merely as dormant spores that have settled on the soil as dust from the air, and have been washed down by rain. Nevertheless, it was H. J. Conn (1922) himself who answered the question he had propounded a few years earlier; he found that fungal mycelium was quite common on smears of soil dried and stained on a glass microscope slide, and since then no one has doubted that fungi are active as mycelia in the soil. The settling of this question was thus the first important result obtained by direct microscopical observation of the soil.

In thus preparing a smear of soil upon a microscope slide, the actual arrangement of soil particles and micro-organisms as it occurs in the soil is destroyed. This loss of information was later avoided by the evolution of a very simple device now known as the *Rossi-Cholodny slide technique*. An Italian microbiologist, G. Rossi (1928), first showed that a "contact picture" of the soil microflora could be obtained by pressing a clean glass microscope slide against an exposed face of soil, "fixing" it by drying or gentle heating, and then staining. The Russian microbiologist, N. Cholodny (1930), then demonstrated that if a number of such slides was allowed to remain in the soil and taken out for examination after selected intervals of days or weeks, then the behaviour of the soil microflora over a period of time could be followed under the microscope. Because of its extreme simplicity and elegance, the Rossi-Cholodny slide has been one of the most valuable techniques ever devised for this purpose, and many special applications have been made for particular problems. It has given most soil microbiologists their first idea of what the soil microflora actually looks like *in situ*. It permits a dramatic demonstration of the effect of adding a small amount of fresh organic material to the soil. One can see the rapid development of fungal mycelia that have arisen by germination of spores in contact with the organic material, and also actinomycete mycelia and colonies of bacteria. Later, as the supply of nutrients becomes exhausted, the fungal mycelia become empty and begin to autolyse (i.e. to disintegrate under the action of their own enzymes); the empty mycelium is outlined by clustering of bacterial cells, which eventually decompose the dead hyphae. Conn (1932) was the first to show how useful the Rossi-Cholodny slide could be for the study of differently treated soils set up in glass tumblers in the laboratory.

Rossi-Cholodny slides give only scanty information, however, about the

different species of fungi occurring in soil. Aseptate mycelium can be recognized as such; the mycelium of some basidiomycetes can be distinguished as such by the presence of clamp connexions at the septa (a clamp connexion is an arrangement associated with the simultaneous division of the two nuclei constituting a dikaryon), but not all basidiomycete species form clamp connexions. Clamp connexions alongside the septa in mycelium of *Serpula lacrimans* are shown in Fig. 6. Sexual organs, though more often oospores, of *Pythium* and other genera of the Oomycetes are sometimes seen on the slides, as are the conidiophores of *Penicillium* and other genera of imperfect fungi. Interesting as such observations may be, however, they are only incidental to the main use to which the Rossi-Cholodny slide has been put. This has been to obtain some idea of the abundance of fungal mycelium in different soils at different times, or following experimental treatments such as addition of acid or lime, or of fresh organic material. Such information is of value only if it can be expressed quantitatively. This was first done by H. L. Jensen (1934–36) in survey of soils in New South Wales; he determined the quantitative frequency of fungal and actinomycete mycelium, respectively, by recording presence/absence of mycelium in a number of random microscope fields under a 2 mm oil-immersion objective on each slide. Such a microscope field could be termed a "microquadrat", inasmuch as it is an application to microbial ecology of the quadrat sampling method employed by vegetational ecologists.

A direct method of a somewhat different kind for determination of the amount of fungal mycelium in the soil at any time was devised by Jones and Mollison (1948). They made up a weighed amount of soil in suspension in melted but cooled agar, and from this prepared films of known thickness with the aid of a haemocytometer slide. When set, the films were removed, dried, stained and mounted for microscopical observation upon another slide; the total length of fungal hyphae was measured, and expressed in terms of the original weight of soil. Various other methods for direct microscopical examination of soil have also been employed. W. L. Kubiena (1938) used a microscope together with strong direct illumination to examine faces of soil *in situ*, outside in the field. He also used a resin for impregnation of undisturbed blocks of soil; when the resin had set, sections were cut for examination. More recently, hard-setting resins have been used for soil impregnation; sections are then prepared by grinding down, as is done for examination of geological sections (Burges and Nicholas, 1961).

The usefulness of these methods of direct microscopical observation has been somewhat circumscribed by their limitations, though they have given information that could have been obtained in no other way. They will continue, no doubt, to be employed as a check upon conclusions derived from cultural methods, but their chief value in the future may well be in functional combination with cultural methods, as will be described later in this chapter.

STUDIES OF FUNGAL COMMUNITIES BY CULTURAL METHODS

For the isolation of special groups, down to genera, of soil fungi, the so-called "baiting methods" have been widely employed by fungal taxonomists. The "bait" consists of a substrate that is more likely to be colonized by species in the desired group than by any others. Thus boiled hemp seed happens to be a good bait for species of *Pythium*, the occurrence of which in Indian soils was first studied, using baiting methods, by E. J. Butler (1907). The baiting method as used by mycologists, however, is merely an application of an older method, the *enrichment culture method*, which was developed independently by S. Winogradsky and M. W. Beijerinck during the latter years of the last century. By this method, the soil is enriched with the energy substrate of the organism it is desired to isolate; other energy substrates are excluded as far as possible, and environmental conditions are made optimum for decomposition of the substrate under study. This procedure results in a large and disproportionate increase in the population of micro-organisms that utilize the particular substrate, so much so that they may outnumber others by 1000 to 1 or more. By such a method, Winogradsky in 1890 finally succeeded in isolating pure cultures of the nitrifying bacteria, *Nitrosomonas* and *Nitrobacter* (Ch. 3). A strictly inorganic culture medium was used so as to exclude competition by heterotrophic bacteria, and it was well buffered against development of metabolic acidity.

Isolation and growth of fungi in pure culture

At this juncture, it is necessary to outline the methods used for isolating and maintaining fungi and other micro-organisms in pure culture, i.e. as populations of a single species. Most of the methods now employed by

mycologists were originally devised for the culture of bacteria, and all of them can be traced back to the methods originated by Pasteur and his immediate successors. In order that fresh culture media can be rendered free of bacteria, fungi and other micro-organisms, they have to be *sterilized* before use. Boiling a liquid is not sufficient for complete sterilization, because the spores of most spore-forming species of bacteria can withstand exposure to 100° C. If, however, a liquid is boiled, and then allowed to cool on 3 successive days, it is usually rendered sterile, because the bacterial spores are usually stimulated to germinate by the first heating, and the active vegetative cells resulting from spore germination are killed by the second boiling; a third sequence of 24 hours incubation followed by boiling makes the method more certain. This technique of *intermittent sterilization*, which was introduced by John Tyndall around 1876, was used when necessary to sterilize a culture medium without heating it above 100°C; higher temperatures accelerate thermal decomposition of some nutrient substances, and can also cause interaction between different constituents of a culture medium to produce toxic substances. Nowadays other methods, such as filtration through heat-sterilized filter-beds that will hold back even the smallest species of bacteria, and sterilization by ultra-violet light or ultra-sonic vibration, are generally used for heat-sensitive culture media. Much the most common method of sterilizing culture media, however, is to heat to a temperature of about 120°C in an *autoclave*, which is the laboratory precursor of the domestic pressure cooker. Steam under a pressure of 1 atmosphere (1.013 bar) is at a temperature of 120°C; exposure of a culture medium to this temperature for 10–30 min. (according to the volume of liquid being treated) is sufficient to ensure sterility. Bacterial spores, and all other forms of micro-organisms, are much more resistant to dry than to moist heat; dry glassware intended for manipulation or storage of culture media therefore has to be exposed to a temperature of about 160°C for a period around 2 hours.

Culture media for fungi and other micro-organisms must contain all the nutrients essential for growth. The so-called "natural" culture media, such as malt extract and decoctions of vegetables (e.g. potato, carrot, runner bean) or fruit (e.g. prune), usually contain all that is necessary for growth; vegetable decoctions are often supplemented with 2% glucose to permit more vigorous growth. "Synthetic" culture media are made up of a sugar (commonly % glucose), together with smaller amounts of a nitrogen source

(nitrate, ammonium or organic), potassium phosphate and magnesium sulphate. This is the simplest and commonest form that a synthetic medium can take; more complicated media have been designed for particular organisms or purposes. Iron, with or without other trace elements, is usually added as a precaution against deficiency, though trace elements other than iron are often present in sufficient amount as impurities in other constituents of the medium. For some micro-organisms, the medium may have to be supplemented with one or more vitamins (most commonly thiamine). Fungi grow best in a slightly acid medium (*ca.* pH 5.5); for bacteria, the medium is made neutral or slightly alkaline.

Fungi, as well as bacteria, will grow perfectly well in a shallow (*ca.* 2 mm) layer of liquid culture medium, because such a layer is sufficiently aerated. Medicine bottles, laid flat on one of their broader sides, are frequently used as culture vessels. For larger volumes of liquid in deeper vessels, forced aeration must be employed; a stream of filtered, sterile air can be blown through the liquid, or the culture vessel can be mechanically shaken or stirred. These are the methods employed for the industrial production of citric acid, penicillin, etc.; on a pilot scale in the laboratory, batteries of domestic washing machines (old paddle type) have been adapted for antibiotic production.

For the great majority of mycological investigations, however, fungi are grown on culture media solidified by the incorporation of a gelling agent. Such solid culture media are more convenient for the handling, examination and storage of cultures. Gelatin was the first gelling agent to be tried and was introduced by the German bacteriologist, Robert Koch. Gelatin, however, changes into a liquid at temperatures above 28°C, and, being a protein, is decomposed and liquefied by a large number of micro-organisms. Another gelling agent, agar-agar (usually known as "agar"), was later suggested by Frau Hesse, the wife of one of Koch's research associates, who thereby performed for microbiology a more enduring service than have most full-time microbiologists. Agar is extracted from the tissues of various red algae and was originally produced for culinary purposes; it is completely resistant to decomposition by all micro-organisms except a few species of bacteria, which most mycologists never see. Agar is incorporated with a liquid culture medium by heating to 100°C; once melted, however, it remains liquid until the temperature falls to about 40°C. This is very convenient, because micro-organisms can withstand,

without apparent harm, a few minutes exposure to 40°C; consequently it is possible to add a suspension of microbial cells to an agar medium first melted and then cooled down to this temperature. If such a suspension of microbial cells in agar is thoroughly agitated and then poured out as a shallow layer in the bottom of a culture vessel, the liquid rapidly cools and gels, thus fixing the microbial cells in position. If the microbial suspension has been properly agitated before pouring, the individual cells are evenly dispersed over the area of the culture vessel, as are the colonies to which they give rise. By making a suitable series of dilutions of the original suspension of microbial cells, a series of agar plates may be prepared so as to give microbial colonies at any desired degree of spacing. This method, which was introduced early in the history of microbiology, is known as the *dilution plating method*, and has been widely used in studies of soil micro-organisms.

In the early days of microbiology, a considerable variety of culture vessels was employed; the contained medium could be kept sterile if the neck of the vessel was plugged with cotton wool before the medium was sterilized; wide vessels were covered with glass plates. In this connexion, a second and equally outstanding contribution to the development of microbiology was made by another of Robert Koch's associates—this time in his own right and not merely as one of the most fortunate of husbands. This second associate was R. J. Petri, and the *Petri dish* that he invented has survived unchanged to the present day, being used by the hundred in every laboratory of microbiology. History records that Petri, whose talent for propitiating those in authority must have been at least as felicitous as his ingenuity, introduced his invention as merely a "slight modification" of his master's much more cumbersome methods. The Petri dish consists of a shallow glass dish of cylindrical form, usually of 9 cm diam., covered with a loosely fitting lid (Plate 1). It is extraordinarily efficient in excluding unwanted microbial cells, so many of which are suspended in the laboratory atmosphere; its remarkable success in actual practice could hardly have been anticipated by Petri, nor by anyone else. In the present age of plastic containers, "disposable" plastic dishes, sterilized by ethylene oxide gas, are widely available; the dish bottom is more uniformly flat than that of most glass dishes, thus providing a non-variable depth of agar. Once-used dishes make useful "moist chambers" for non-sterile purposes.

The soil dilution plate

This method was originally developed by soil bacteriologists, for whom it was merely a special application of their well-established dilution plate procedure for the isolation of single species of bacteria in pure culture. In brief, the method consists of shaking up a known weight of soil in sterile water or saline, either by hand or mechanically and sometimes with the addition of glass beads to facilitate the breaking down of soil aggregates, and then progressively diluting with the sterile shake medium. From one or more of the higher dilutions, 1 ml samples are taken and dispersed with a standard volume of cooled but still liquid nutrient agar in a Petri dish. For the isolation of soil fungi, a range of soil dilutions lower than that used for bacteria is employed, because population "numbers" of soil fungi are lower. A nutrient medium particularly suitable for the development of fungi is selected. It is also necessary to make this medium as unsuitable as possible for bacteria, so that bacterial colonies do not interfere with the development and counting of fungal colonies. The earliest method for suppressing bacteria was to make the medium too acid (pH 4.0) for their growth. This method is still employed, though some mycologists now prefer to incorporate one or more anti-bacterial antibiotics, such as streptomycin, with the medium. Another bacteriostatic agent that has been widely used, both by itself and in conjuction with antibiotics, is rose bengal, which was originally proposed for this purpose by Smith and Dawson (1944). Rose bengal has the further advantage that it slows down growth of fungal colonies, and reduces the tendency of fast-growing fungi to spread quickly over the whole plate before more slowly growing fungi have had an opportunity to form colonies.

Soil dilution plates have been much employed by mycologists for the comparison of "soil fungus floras" in different soils, in the same soil after different agricultural treatments such as addition of organic manure or lime, and in the same soil at different seasons of the year. Different species of fungi can be identified and the frequency of their occurrence in any particular sample of soil can be expressed numerically. The method has also been much used for estimating total populations of fungi, bacteria and actinomycetes, as a criterion of microbial activity in any soil at one particular time.

The dilution plate method suffers from several disadvantages. First of all

there is the general disadvantage, common to all such cultural methods, that no single culture medium is optimum for the development of more than a small minority amongst species of soil fungi; this statement applies equally well to bacteria and actinomycetes. Amongst species of soil fungi that are rarely found on soil dilution plates, only a small proportion fails to appear because the culture medium is nutritionally inadequate. Some species are not represented because their population numbers are too low by comparison with those of the common species; the probability that a viable propagule of an uncommon species may be included in a sample plated from the highest dilution is not nil, but it may be extremely low. Many species quite common in soil, however, also remain largely unrepresented on the dilution plate; the reason for this is that development of their propagules is inhibited at an early stage by the competition of faster-growing fungi, and so these more slowly growing fungi are unable to form visible colonies. Species of the higher Basidiomycotina, which are common in soil but grow rather slowly, are rarely found on soil dilution plates.

The second disadvantage of the soil dilution plate is also common to the isolation of bacteria and actinomycetes, but it operates more significantly on the isolation of fungi. This disadvantage arises directly out of the procedure for diluting the original suspension of soil. Even before a sample of soil suspension has been completely pipetted off to make a dilution in fresh shake medium, most of the heavier part of the soil has already sunk to the bottom of the shaking vessel; this part will include the larger mineral particles, together with those soil crumbs that have not been disaggregated by the shaking procedure, and the heavier organic fragments. The actual samples that are finally plated out in the Petri dishes are therefore suspensions of the finer soil particles, together with bacterial and actinomycete cells, fungal spores and some hyphal fragments. In such a suspension, viable fungal spores greatly outnumber viable hyphal fragments; any fungus that is sporing heavily in the original soil sample will therefore tend to be represented on the dilution plate at a frequency out of all proportion to the volume of its active mycelium. The soil dilution plate is a reasonably satisfactory method for the counting of numbers of soil bacteria, because these are unicells; it is also adequate for actinomycetes, because their hyphae fragment very readily into individual hyphal cells. But for fungi, the count of "numbers" is rendered almost meaningless by the

somatic dichotomy of these organisms into vegetative hyphae and spores. What is wanted, as a population statistic, is some estimate of the total volume of viable microbial protoplasm in a given sample of soil. The dilution plate gives a first approximation to such an estimate for bacteria—but not for fungi.

Dr. J. H. Warcup, who has made some notable contributions to the ecology of soil fungi, has interested himself particularly in this important question of techniques, and has made a special study of the nature of the propagules giving rise to fungal colonies on soil dilution plates (Warcup, 1955*a*, 1957). By direct microscopical observation of very young fungal colonies developing on soil dilution plates, Warcup was able to identify the point of origin for about 95 % of the colonies. Of these, some 75 % were found to arise from spores, 20 % from within humus particles and 5 % from visible fragments of hyphae. By a quite simple procedure, Warcup was thus able to settle a question about which hundreds of pages had already been penned by mycologists. More than this, he effected a fusion between the methods of culture and of direct observation, which points the way to further development of combined methods.

Despite its obvious disadvantages, the soil dilution plate has provided much useful information. Provided that its limitations are recognized, it is still the best method for some purposes. After partial sterilization of soil by steam or fumigants, for instance, surviving fungal propagules will consist chiefly of spores, because these tend to be more resistant to lethal agents than are growing hyphae. For investigation of the fungal flora immediately surviving partial sterilization, the soil dilution plate has much to recommend it. If the number of colonies developing on the plates poured at the highest dilution is kept down to about 25 per plate (9 cm diam.), then the degree of competition between developing colonies will be less than that occurring with any of the remaining techniques to be described. Sources of variability in the soil dilution plating method for fungi have been thoroughly studied by D. Hornby (1969).

The Warcup soil plate

This was designed by Warcup (1950) in order to reduce some of the particular disadvantages from which the soil dilution plate suffers. By Warcup's method, small samples (0.005–0.015 g) of soil are taken with the

flattened blade of a sterilized nichrome inoculating needle, which is then used to crush and disperse the soil aggregates in the bottom of a sterile Petri dish; a little sterile water is added to assist in the dispersion of soils that do not break up easily. Melted and cooled agar (8–10 ml) is then poured into the dish and manipulated before setting so as to secure as complete as possible a dispersion of the soil particles.

Since its introduction, the Warcup soil plate has been widely used; much of its popularity is due to the fact that a series of soil plates is much less tedious to prepare than is a series of soil dilution plates. By incorporation of the whole of the soil with the agar, the method permits isolation of fungi that are rejected with the soil residue by the dilution plate method, and so the soil plate has at least the potentiality of isolating a wider range of species than that obtained with the dilution plate. The soil plate method reduces, though it does not eliminate, the advantage obtained by heavily sporing fungi. Inter-colony competition, on the other hand, is certainly more severe than on a dilution plate giving not more than 25 colonies, and here the soil plate is at a disadvantage. Warcup (1960) has himself provided a comparative survey of the results obtained with this and other methods.

Chesters's soil immersion tube

With this method and the next one, the procedure adopted in the soil plate and dilution plate methods has been reversed; instead of incorporating a small quantity of soil or soil suspension with an agar plate, a corpus of nutrient agar is exposed to colonization by soil fungi in the soil actually *in situ* in the field. As described by C.G.C. Chesters (1940, 1948), the method consists in the immersion in soil of a glass tube with four to six spirally arranged, invaginated capillaries providing entries into the tube from the soil outside. The whole tube is filled with a nutrient agar; fungi growing up the capillaries can later be isolated from the central core of agar.

Chesters designed this method for the purpose of isolating actively growing mycelia from the soil. It now seems extremely doubtful whether the method does discriminate between active mycelium and spores, because the spores of fast-growing fungi germinate very rapidly in contact with a substrate, and soon overtake the mycelia of more slowly growing fungi. But because a fungus has to grow some distance from the orifice of one of the invaginated capillaries to reach the cylindrical core of agar (which is later

cut out with a cork borer down the centre of the tube and plated out in segments), the method tends to select fast-growing fungi. Because the oxygen tension within this core of agar is low, the method further tends to select fast-growing fungi that can tolerate a low oxygen tension. Thus the common and fast-growing fungus *Trichoderma viride* agg. is rarely isolated from these immersion tubes; in my own laboratory, we have found this fungus to be intolerant of poor soil aeration. The really important contribution to knowledge made through these immersion tubes has been to show that the list of commonest soil fungi isolated by this technique is very different from that given by the soil dilution plate. The immersion tube has been particularly successful in isolating mycelia of common soil fungi that are either non-sporing (e.g. *Rhizoctonia solani*) or sparsely sporing (e.g. *Pythium* spp.). A simplified version of this soil tube was devised by Mueller and Durrell (1957) and can be recommended as easier both to make and to use.

Thornton's screened immersion plate

This method, as described by R. H. Thornton (1952), employs a glass microscope slide coated with a thin layer of water agar, which is enclosed in a Perspex box with ten spaced holes in the lid through which fungi enter and colonize the adjacent agar. After a sufficient period of burial in soil, the slide is examined under the microscope and fungal colonies are subcultured onto plates of nutrient agar for subsequent identification. In their comparative study of a number of methods for isolation of soil fungi, Chesters and Thornton (1956) found that the species lists given by the screened immersion plate agreed more closely with those obtained from immersion tubes than with those given by any of the other methods tested. But, by comparison with the immersion tube, the screened immersion plate reduced the particular advantage given to fungi tolerant of low oxygen tension in the culture medium. In these trials by Chesters and Thornton, the screened immersion plate isolated a wider range of species than was obtained with any of the other methods. Part at least of this success may be due to Thornton's choice of plain water agar instead of nutrient agar as a coating for the slides. Water agar is much less selective than a nutrient agar, because mycelia do not grow strongly enough to produce concentrations of excretory products inhibitory to neighbouring mycelia, and so the intensity

of competition is much reduced. Other workers have also used water agar as their original isolation medium in conjunction with other methods; this choice has not been popular, however, because fungi growing on water agar do not so readily produce identifiable fruiting structures, for which a certain threshold concentration of nutrients is required. This means that some mycelia must be subcultured to a nutrient agar for identification, and thus entails extra work. Nevertheless, the results of Chesters and Thornton suggest that a good return is obtained from this additional labour.

Conclusions on cultural methods

The foregoing account describes those cultural methods that have been most widely used. Various modifications of these methods have also been employed, and a fuller account is given by Warcup (1960). The evolution of these different methods has been prompted in part by the hope of finding the ideal method, which theoretically would be capable of isolating almost all species of soil fungi. Most mycologists must have concluded by now that this ideal method is impossible of achievement, and that complete knowledge can be attained only through the combined use of a number of complementary methods, which should include direct microscopical observation as well as culturing. In the meantime we can conclude that it is the differences rather than the similarities between the lists of typical species given by the different cultural methods that have provided the most useful information.

METHODS COMBINING CULTURING WITH DIRECT MICROSCOPICAL OBSERVATION

As indicated above, Warcup has been a pioneer in the development of the combined method, which he first employed in an attempt to determine the nature of the fungal propagules giving rise to colonies on his soil plates (Warcup, 1951). Later he carried out more ambitious analyses of the origins of colonies on soil dilution plates (Warcup, 1955*a*, 1957). This enabled him to defeat an earlier criticism of current methods, when I remarked that by cultural methods one identified what one couldn't see (i.e. *in situ*), whereas by direct methods one saw what one couldn't identify (Garrett, 1952).

Tribe's Cellophane method

This extremely elegant method, as devised by H. T. Tribe (1957, 1960), is an organic combination rather than a mere welding of the cultural and direct observation methods. In essence, Tribe replaced the Rossi-Cholodny slide with a thin, transparent film of organic substrate, of strictly defined composition. The substrate he selected was the cellulose film marketed as a wrapping material under the name of "Cellophane", by the British Cellophane Company. After boiling and washing to remove plasticizers, the Cellophane is cut up into 22 mm squares and mounted on glass coverslips of the same size before burial in soil. As the cellulose has been altered by the process of conversion into Cellophane, Tribe's method does not provide a critical test for the ability of a particular species of microorganism to decompose natural or "native" cellulose; for this purpose, filter paper or cotton wool is preferable.

The chief virtue of Tribe's method is that it provides an actual picture of the microbial succession developing on a defined substrate buried in soil. Fungi are the first colonizers; the main development of bacteria comes later. Zones of enzymic erosion of the Cellophane are clearly to be seen around some of the fungal hyphae. Micro- and meso-fauna also develop on the Cellophane, and the activity of mites in devouring fungal mycelium can be observed. This is highly educative for botanically trained microbiologists who are apt to forget that the soil fauna affects their studies. Some of the fungi produce fruiting structures, from which they can be identified, on the Cellophane; sterile mycelia can be cultured and possibly identified later. In general, Tribe's technique could be employed with any substrate, such as chitin or gelatin, that can be prepared as a thin, transparent or translucent film.

METHODS DESIGNED FOR ISOLATION OF SELECTED COMPONENTS OF THE SOIL FUNGAL FLORA

Up to about 1950, much of the work on soil mycology could be criticized on the grounds that the culturing of soil fungi was carried out in too general a way, and that little attention was paid to the actual substrate on which any particular species was growing. Since that time, mycologists have become much more aware of the importance of substrate distribution

within the soil; they have studied fungal colonization of selected substrates by means of techniques specially designed for the purpose.

Warcup's hyphal isolation method

Although this technique (Warcup, 1955*b*, 1957) is not concerned with fungi colonizing any particular substrate, it is a selective method and hence appropriately considered here. It was designed for the isolation of fungi occurring in soil as sterile mycelia; Warcup's studies of the dilution plate method have shown that such fungi have a chance of less than 5% of forming colonies on the dilution plate. Such fungi, when isolated, can be characterized and described; whether they can be identified as species depends upon whether they can be induced to form fruiting structures in subsequent culture. The isolation procedure is as follows. A soil crumb is allowed to become saturated with water and is then broken up with a strong jet. The heavier particles are allowed to sediment and the finer particles are poured off in suspension. The process of washing is repeated, until a residue consisting of heavy particles alone remains; this roughly corresponds with what is left behind by the dilution plate method. These residual particles of soil are then spread out in a thin film of sterile water, and searched under the stereoscopic dissecting microscope for fungal hyphae, which are picked up with sterile forceps and plated out on nutrient agar. The list of species isolated by Warcup with this method was very different from that obtained with the soil dilution plate for the same sample of soil. Many of the species obtained by hyphal isolation were non-sporing, and remained sterile in culture.

Method of Harley and Waid for root-surface fungi

The root surfaces of higher plants are occupied by an epiphytic flora of fungi (as well as one of bacteria), certain species of which are characteristic for this habitat. The substrate is provided chiefly by root exudates. For the identification of root-surface fungi, it is essential to separate them from rhizosphere fungi. Harley and Waid (1955) achieved this by serial washings of root segments in changes of fresh sterile water until all or nearly all the soil had been removed. Previous investigators also had done this; the valuable new feature of the method introduced by Harley and Waid was the

inclusion of a check on the efficiency of washing, through the plating out of samples of the wash water. Results showed that the limit of efficiency of serial washing in removing nearly all fungi, except the actual root surface inhabitants, was reached at about the 20th washing.

The same serial washing technique was further applied by Harley and Waid to a study of the fungi colonizing petioles of fallen beech leaves. The method was taken up by Parkinson and Kendrick (1960) for following fungal decomposition of pine-needle litter and of couch grass residues buried in the soil. Then Parkinson and Williams (1961) described an apparatus whereby soil can be washed free of fungal spores (in 25–30 washings) and separated into four categories: organic fragments and three size fractions of mineral particles. By this technique, it has proved possible to isolate hyphae attached to mineral particles or embedded in organic fragments. Some of the species thus obtained have been non-sporing forms that would have been missed by other cultural methods.

Substrate-baiting methods

These are the methods most widely used for cultural isolation of selected components of the soil fungus flora. Such methods have been employed for isolation and study of keratinophilic fungi, which are widely distributed in soil and are responsible for decomposition of keratin in bird feathers, animal hair, and human nail-clippings (Griffin, 1972). Such fungi are usually slow-growing and hence do not appear on soil plates with sugar as a carbon source. Soil-baiting with cellulose or with cellulosic substrates, such as mature-plant tissues, encourages development of cellulolytic fungi, as does the use of Cellophane film by Tribe's method (above); the regular occurrence of *Rhizoctonia solani* on these films led to recognition of its widespread occurrence as a cellulolytic fungus in soil (Garrett, 1962; Bateman, 1964). The most direct method for recording the occurrence of such cellulolytic fungi is the *cellulose-agar plate*, devised by Eggins and Pugh (1962). The carbon source is provided by 1 % ball-milled, filter-paper cellulose, supplemented by essential mineral salts and vitamins. Growth of cellulolytic fungi from soil inocula is revealed by local clearing of this white, opaque agar medium and becomes apparent after 5–7 days at 25°C.

The foregoing survey of techniques employed in the general study of soil fungi has not been exhaustive, but can be supplemented by reference to the

compilation by Parkinson, Gray and Williams (1971) in their *Methods for Studying the Ecology of Soil Micro-organisms.* I shall also describe some further special techniques as they become relevant in following chapters.

CHAPTER 7

COMPETITIVE SAPROPHYTIC COLONIZATION OF SUBSTRATES BY SOIL FUNGI

A substrate for a soil fungus consists of a corpus of organic material, and of exudates from it, which provides the fungus with both a supply of energy for growth and metabolism and with nutrients needed for synthesis of protoplasm. The most widespread substrates for fungi are those provided by higher plants. The exudates from young and active roots are an immediately available nutrient solution of sugars and amino acids. A corpus of dying or dead plant tissue, such as a leaf, twig or root, may contain some free sugar and some starch but the bulk of the carbon compounds available for energy supply are located in the cell walls of the tissues, in the form of pectic substances, hemicelluloses, cellulose and lignin. Young plant tissues contain sufficient mineral nutrients for their complete decomposition but degradation of mature tissues is rate-limited by the supply of mineral nutrients, and especially by that of nitrogen, which is required in largest amount for synthesis of microbial protoplasm. Such mineral deficiencies may be rectified, wholly or in part, by inwards diffusion from the surrounding soil. But rate of decomposition of a massive corpus of woody tissue, such as a large tree-root, is usually restricted by nitrogen poverty of the tissue. So the term "substrate" is used in a general sense to denote a corpus of organic material, or of exudates from it, on which a fungus is subsisting. But the term is also employed, in a narrower sense, for a particular carbon constituent of a tissue from which a fungus is deriving its supply of energy; such a usage is parallel to that of the term "substrate" for a particular enzyme.

The spectrum of carbon constituents in plant tissue that is available to a particular fungal species is a primary determinant of its ecological niche in the soil economy. But the share of a potential substrate that such a fungus will actually obtain by competition in the soil will always be smaller than the proportion that it can exploit in pure culture in the laboratory. During colonization and exploitation of a substrate, the most quickly available carbon-source, i.e. sugars, will be used up first, whereas cellulose and lignin will persist longest. Fungi will be accompanied by bacteria, protozoa and members of the soil micro-fauna but will play their usual part of pioneer colonizers unless the substrate is too wet and thus too poorly aerated for them; in such a situation, fungi will be replaced by bacteria. As colonization proceeds, the substrate will be changed by progressive exploitation and final exhaustion of the various carbon constituents of the plant tissue; these changes will be reflected by a succession of different fungal species as dominant decomposers in consecutive phases of tissue degradation.

FUNGAL SUCCESSION ON A SUBSTRATE

Fungal succession on a substrate is more difficult to demonstrate than might be supposed because most of the mycelium within a corpus of plant tissue buried in soil is in a sterile state and so cannot be identified by direct microscopical observation; the same difficulty also occurs in direct identification of mycelia amongst soil crumbs, as noted in Chapter 6. So indirect methods for identifying mycelia within the substrate often have to be used and may bias the observations. Nevertheless, from the work reported on fungal successions we can construct at least a general schema.

In the schema below, *Stage 1a* represents colonization of senescent tissues by weak parasites. Senescent leaves, twigs and roots all possess at least some residual degree of resistance to invasion by fungi and other micro-organisms and this residual resistance exercises a selective effect upon the fungal species that become the first invaders. Because such tissues are still alive, though senescent, so the fungi colonizing them must by definition be regarded as *parasites*. Although the above schema, as constructed for the first edition of this book, probably remains broadly representative of current views, yet the balance of emphasis has changed since 1963. Thus *Stage 1a* is considered to be more important than formerly

Senescent tissue	Dead tissue		
Stage 1a	*Stage 1*	*Stage 2*	*Stage 3*
Weak parasites	Primary saprophytic sugar fungi, living on sugars and carbon compounds simpler than cellulose	Cellulose decomposers and associated secondary saprophytic sugar fungi, sharing products of cellulose decomposition	Cellulose and lignin decomposers and associated fungi

————General trend of fungal succession————→

believed. At least in moist climates, leaves, fruits and twigs of plants are likely to be at least partially colonized in senescence even before they reach the ground. Work on this subject has been broadly reviewed by H. J. Hudson (1968) in a paper entitled "The ecology of fungi on plant remains above the soil". As a common practice to maintain soil organic content, farmers plough in a "catch crop" of legumes or other quick-growing crop. Such immature leafy tissues are often colonized by parasitic species of *Pythium* and *Rhizoctonia*, which are common pathogens of seedlings and of senescent tissues but are usually unable to invade mature-plant tissues in full vigour. In situations of disease-risk, therefore, it will be safer to plough in dead, mature crop residues rather than a green-manure crop (Garrett, 1970, pp. 48, 139).

Stage 1 of the fungal succession consists predominantly of *saprophytic sugar fungi*, a grouping proposed by Thom and Morrow (1937) and elaborated by A. Burges (1939); they are defined as fungi living on sugars and the simpler carbon compounds but unable to degrade cellulose or lignin. Sugar fungi are fast-growing species, and are commonest amongst the Oomycetes and Zygomycetes. Sugars are the most ephemeral carbon

constituent of green plant tissues; they are consumed and quickly exhausted by the pioneer fungal colonizers. Thus a sugar-based nutrient agar placed in contact with soil in a Chesters soil-immersion tube tends to be colonized first by fast-growing sugar fungi (Ch. 6).

Stage 2 of the fungal succession, comprising cellulose-decomposers and associated fungi, has been studied particularly in compost heaps of organic material. Cultivated mushrooms (*Agaricus bisporus*) were formerly grown on composts of stable and farmyard manures. With decrease in production of such manures and increase in cultivation of mushrooms, growers turned to composts of wheat straw supplemented with mineral nutrients needed for quick decomposition. The sugar content of mature wheat straw is low; Chang and Hudson (1967) demonstrated a brief phase of primary saprophytic sugar fungi, chiefly zygomycetes, which were followed by ascomycetes and imperfect fungi, and finally by basidiomycetes of *Stage 3* in the succession. Such a succession can be regarded as a nutritional one, with decomposers of cellulose and lignin constituting the final fungal phase. This is recognized in practice by mushroom growers, who wait until the compost has reached the third stage of fungal succession before inoculating it with the mushroom "spawn". During making of the compost, early stages in its development are characterized by violent microbial activity, during which most of the carbon constituents simpler than cellulose are metabolized. This microbial combustion is accompanied by evolution of heat, so that the interior of the compost heap may reach a temperature of *ca.*75°C. Under these conditions, thermophilic cellulose-decomposers, such as *Chaetomium thermophile* with an optimum growth temperature around 50°C, are active. But the mushroom fungus is not a thermophile and can tolerate neither the microbial competition nor the high temperature that are characteristic of the heating phase of compost preparation. By mixing and turning the compost heap at intervals, the grower ensures that all of the straw has passed properly through this first stage of composting. Not until composting has been completed does the grower inoculate the bed with mushroom spawn; then, but not until then, the spawn will "run" properly and colonize the compost, in preparation for the final production of fruit-bodies.

An interesting ecological niche in *Stage 2* of the fungal succession is occupied by the fungal group that I originally designated as *secondary saprophytic sugar fungi* in the first edition of this book in 1963; such fungi

were there defined as non-cellulolytic but able to live commensally alongside cellulose decomposers, taking their share of the sugars (cellobiose and glucose) liberated during cellulolysis. Commensalism of this type was demonstrated by H. T. Tribe (1966) for *Pythium ultimum*, *P. debaryanum* and *P. oligandrum* by means of his cellulose-film technique (Ch. 6). These three species grew poorly on cellulose film by themselves and produced very few oogonia, numbers of which were countable and so afforded a quantitative estimate of mycelial growth. But when grown on the film in association with certain cellulolytic fungi, such as *Fusarium culmorum*, all three *Pythium* species grew vigorously and produced large numbers of oogania. Although *Rhizoctonia solani* decomposed cellulose film faster than *F. culmorum*, yet it supported less growth of the *Pythium* spp. than did *F. culmorum*. From this observation and from later ones on other fungal species, it is evident that a non-cellulolytic fungus like these species of *Pythium* cannot live as a commensal alongside all cellulolytic fungi; a commensal relationship can be established only with compatible "host" fungi. Neither *P. ultimum* nor *P. debaryanum*, both of which cause seedling diseases, has been observed as a destructive parasite of other fungal mycelia. So their relationship with *F. culmorum* on cellulose film appears to have been purely commensal. As Tribe (1966) noted, however, *P. oligandrum* is known to attack the mycelia of other fungi as a destructive parasite. J. W. Deacon (1976) later reported it as pathogenic to both *Fusarium culmorum* and *Botryotrichum piluliferum*, and it greatly reduced the rate at which they decomposed filter-paper cellulose. Such quantitative measurements of cellulose decomposition provide unequivocal evidence of pathogenesis and are more reliable than microscopical observations. Thus one fungus may be pathogenic to, and parasitic upon, the mycelium of another through production of a lytic toxin; in such a case, parasitism is external and not accompanied by invasion of host hyphae.

Commensalism of this type occurs among thermophilic fungi during the heating phase of wheat-straw composts, during which the non-cellulolytic *Thermomyces lanuginosus* was found by Hedger and Hudson (1974) to be prevalent. The commensalism of *T. lanuginosus* on sugars produced by two strong cellulose-decomposers, *Chaetomium thermophile* and *Humicola insolens*, respectively, was demonstrated by means of paired cultures on filter-paper. Hedger and Hudson showed that this association with *T. lanuginosus* did not significantly affect rate of cellulose decomposition by

either *C. thermophile* or *H. insolens*, thus excluding any possible pathogenic action of *T. lanuginosus*. Further evidence for commensal uptake of sugar by *T. lanuginosus* was provided by estimation of reducing sugars in the culture fluid, the level of which was significantly reduced in paired culture with *T. lanuginosus* when compared with that in pure cultures of either cellulolytic fungus by itself. But when paired in turn with two other cellulolytic fungi isolated from compost, *T. lanuginosus* was unable to establish a commensal relationship, thus recalling the earlier finding of Tribe (1966).

Although primary and secondary sugar fungi share a common nutritional restriction in the substrates on which they can live, yet they occupy rather different ecological niches in the general fungal succession. As pioneer colonizers, primary sugar fungi are not exposed to high levels of fungistatic substances because these are not produced as secondary metabolites until the substrate has been occupied completely. So pioneer colonizers are not necessarily qualified for this niche by any notable degree of tolerance towards fungistatic substances and antibiotics. Secondary sugar fungi, in contrast, occupy a niche in which vigorous microbial activity and cellulolysis is proceeding; this is especially evident during the heating phase of composts. We should therefore expect such fungi to be qualified for this habitat by a sufficient tolerance of fungistatic substances produced during microbial degradation of the substrate. Sugar fungi are also to be found as root-surface inhibitants, living on the exudates produced by young and active roots. This is a habitat intermediate between those typically occupied by primary and secondary sugar fungi, respectively.

So far we have discussed only a generalized schema for a nutritional fungal succession on a substrate of virgin plant tissue, during which the admitted overlapping of successive stages does not invalidate the general concept of a succession of fungal groups. But substrates more complex than this occur on and in the soil; a good example of this is the classical laboratory demonstration provided by incubating dung of a horse or other herbivore within the humid atmosphere provided by the old-fashioned bell jar. Fungal fructifications usually oblige by appearing in the following order: zygomycetes, ascomycetes and imperfect fungi, basidiomycetes. For many years, this was provided for classes of students as a nice example of nutritional succession of fungi on a substrate. This interpretation has now

been made untenable through the work of J. Webster (1970) and his associates. It is now established that fungi comprising all stages of the apparent succession commence mycelial activity together, soon after the dung has been deposited. The apparent succession merely reflects the time taken to produce recognizable fructifications, which increases in the order zygomycetes, ascomycetes and imperfect fungi, basidiomycetes.

Fructifications of coprophilous (dung loving) fungi are produced in succession upon dung lying in a sufficiently humid atmosphere on the ground. Spores of these fungi, often violently ejected from their sporocarps, are deposited upon surrounding herbage, with which they are ingested by grazing animals. The spores of these specialized fungi not only survive passage through the animal's digestive tract but are also rendered ripe to germinate, which they do, rapidly and synchronously, after the dung has been voided and has become sufficiently aerated. Herbivore dung is thus a complex substrate, consisting of herbage chewed into fragments by the grazing animal and then mixed with animal secretions and partially digested by the anaerobic microflora of the digestive tract, amongst which cellulolytic bacteria are especially important. Loss of cellulose by this digestion increases the proportion (*ca.*4 %) of nitrogen in the dung to three or four times that in the original herbage, so that rate of further decomposition of the dung is not limited by shortage of nitrogen; it will depend chiefly on maintenance of a sufficient moisture content and on the ambient temperature. So when these environmental conditions are favourable for continued microbial activity, fungi are operating in a site of intense competition. It was in dung that Ikediugwu and Webster (1970) discovered the remarkable phenomenon of *hyphal interference*, as shown by the coprophilous fungus, *Coprinus heptemerus*. This is a very short-range antibiotic effect, not mediated by an antibiotic diffusing out from the producing hypha but occurring only when the attacking hypha of *C. heptemerus* and that of its victim are in actual or proximal contact. Various other fungi are now known to be capable of such hyphal interference, including *Peniophora gigantea* as shown by Ikediugwu, Dennis and Webster (1970). This fungus is widely employed by the U.K. Forestry Commission for covering (with a suspension of oidia spores) the freshly exposed surface of pine stumps after tree felling. This protects the stumps from invasion by *Fomes annosus*, a fungal pathogen causing widespread killing and butt rot of conifers. This protection was devised by J. Rishbeth

(1963) and represents the first method of biological control by inoculation to have become generally adopted in commercial plantation practice. So what was discovered as a phenomenon of purely scientific interest has now turned out to be of great practical importance too.

Fuller details of the ecological assemblages of fungi so far discussed, and of others too, can be found in H. J. Hudson's (2nd. ed. 1980) *Fungal Saprophytism*, which is recommended for further reading.

FACTORS CONTROLLING COMPETITIVE SAPROPHYTIC COLONIZATION

This is a subject that we have been studying in the Cambridge Botany School since 1950. The work has been done with various pathogenic root-infecting fungi and particularly with a group of five species causing foot- and root-rots of wheat and other cereals. Our practical objective was to apply our eventual understanding of the saprophytic life of these fungi in soil towards restricting it through appropriate measures of crop husbandry, e.g. crop rotation, cultivation practices, judicious use of chemical fertilizers and so on. Some of these fungi can compete well with obligate saprophytes in colonization of wheat straw ploughed under the soil after harvest. Their competitive efficiency in saprophytic colonization of straw tissue has been studied by a succession of my research students: F. C. Butler (1953*a*), R. L. Lucas (1955), R. C. F. Macer (1961*a*) and J. W. Deacon (1973). A complementary study of competitive colonization of nutrient agar was made by A. S. Rao (1959) and R. L. Wastie (1961). Fuller details of the work on straw colonization are given by Garrett (1975). Saprophytic *survival* in wheat straw colonized by these fungi will be the subject of Chapter 8.

Success in competitive colonization of a substrate by any particular fungal species appears to be determined by the following factors:

(1) Directly by its *competitive saprophytic ability*, an intrinsic characteristic of the species.

(2) Directly by its *inoculum potential* at the surface of the substrate.

(3) Inversely as the aggregate inoculum potential of competing fungi.

I first proposed the term *competitive saprophytic ability* in 1950 and in 1956 defined it as *the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates*. In 1950 I

suggested four fungal characteristics as likely to contribute to competitive saprophytic ability:

(1) Rapid germination of fungal propagules and fast growth of the young hyphae towards a source of soluble nutrients.

(2) Appropriate enzyme equipment for degradation of carbon constituents of plant tissues.

(3) Excretion of fungistatic and bacteriostatic growth-products, including antibiotics (active at low concentrations).

(4) Tolerance of fungistatic substances produced by competing microorganisms.

This list was as comprehensive as I could make it at that time, nor need I add to it now. But concepts evolve with further research, just as techniques do. I had originally visualized this concept as one embracing most, if not all, situations of saprophytic competition, and that any fungal species could be labelled as strongly, moderately or weakly competitive. But our results have shown that such a classification is valid only for a particular, defined type of carbon substrate. Thus a sugar fungus that is strongly competitive for a sugar substrate has no competitive ability at all for a substrate of pure cellulose, which it cannot decompose even in axenic culture. Conversely, a strong cellulose-decomposer may have only poor competitive ability for a sugar substrate if its growth rate is low, as is that of many basidiomycetes.

The second determinant of success by a fungus in competitive colonization of a substrate is its *inoculum potential*, which I originally defined (Garrett, 1956) as *the energy of growth of a fungus available for colonization of a substrate at the surface of the substrate to be colonized.* The energy of growth of a fungus *per unit area of substrate* is made up of three components:

(1) The number of hyphal apices per unit area, i.e. their density. These may occur in a vegetative mycelium or arise from a population of spores germinating in response to a nutrient stimulus from the substrate. Alternatively the apices may be aggregated together in a mycelial strand, or be organized into the apical meristem of a rhizomorph, or arise from a germinating sclerotium (Ch. 4).

(2) The nutritional status of the hyphal apices, which in mycelial inocula is determined by that of the food-base from which the mycelium has arisen. Inoculum potential of the apices usually varies inversely with distance from

the food-base, because it is limited by rate of translocation (Ch. 4). Vigour of germ-tube growth from a population of spores is determined by their level of endogenous food-reserves, which in turn depends upon the nutrient status of the mycelium producing them. Vigour of germ-tube growth usually declines with progressive ageing of dispersal spores but this effect occurs much more slowly in a population of resting spores or sclerotia.

(3) Environmental conditions around the substrate to be colonized. Inoculum potential is defined as the energy of growth *actually in progress* from the inoculum onto the substrate and so is very dependent on soil conditions around the substrate. So for any body of inoculum, the actual, realized energy of fungal growth from it can vary from a maximum, under optimal soil conditions, down to zero under conditions inhibiting fungal growth, e.g. too low or too high a temperature, an air-dry or a water-logged (and thus anaerobic) soil, or soil at extremes of pH.

The third determinant of competitive success by a fungus in inter-fungal competition for a substrate is the collective inoculum-potential of other fungi; as this increases, so degree of success by a particular fungal species under consideration must be reduced. In order to simplify this discussion of a complex situation, I have restricted it to competition between fungi. In our experimental work next to be described, we arranged for type of substrate and environmental conditions to be favourable for fungal rather than bacterial colonization. But if, for example, we had replaced a soil at medium moisture content with a wet soil, then conditions would have become favourable for bacterial, at the expense of fungal, activity. Wet filter-paper inoculated with crumbs of a fertile cultivated soil becomes colonized by bacteria rather than fungi, as Henis, Keller and Keynan (1961) have shown; on wet filter-paper overlying agar containing mineral salts, cellulolytic bacteria (mainly *Cellvibrio* spp.) developed. But if chloramphenicol (an anti-bacterial antibiotic) was incorporated with the agar, then fungal colonies developed from most of the soil crumbs. Similarly, if circles of *unsterilized* filter-paper are saturated with a mineral-salts solution and incubated in unsterile, plastic Petri-dishes, there is a rapid growth of bacteria but only very occasional colonies of fungi develop from air-borne spores in the laboratory atmosphere; a plate of potato-dextrose agar thus exposed soon becomes covered by fungal colonies (Garrett, 1980).

THE CAMBRIDGE METHOD

The basic idea of this method was to test the ability of a fungal species to colonize a selected substrate when its inoculum potential in unsterilized soil was varied over a wide range. Pure cultures of the inoculant fungus were grown, usually for *ca.* 4 weeks, on a mixture of quartz sand with 3 % (*w*/*w*) maizemeal added; a grown culture breaks down easily into its constituent particles and so can be intimately mixed with natural (i.e. unsterilized) soil. The full range of inoculum-soil mixtures contained the following percentages (*w*/*w*) of inoculum: 100 (pure-culture alone), 98, 90, 50, 10, 2 and 0 (soil alone). The substrate was then offered for colonization to this range of inoculum-soil mixtures; conditions were so arranged as to favour development of fungi rather than that of bacteria. The proportion of substrate colonized by the inoculant fungus was then assessed, after a suitable incubation period, over the whole range of inoculum-soil mixtures tested. Two substrates were tested in turn by the Cambridge method (1) autoclaved units of wheat straw (2) nutrient agar with 3 % sucrose as the carbon source. Although nutrient agar was the second substrate tested, results with it will be described first, as being simpler to interpret.

Nutrient agar as the substrate

This variant of the method was developed by A. S. Rao (1959) and R. L. Wastie (1961) and was used with fourteen pathogenic root-infecting fungi. The various inoculum-soil mixtures were spread as a layer, 1.0–1.5 mm deep, over the bottom of a Petri dish and impregnated with water agar, cooled down to *ca.* 40° C. Disks (4 mm diam.) of the agar-impregnated mixtures were set out (four to a plate) on five replicate plates of modified Czapek-Dox + 0.05 % yeast-extract agar, with 3 % sucrose as carbon source. Bacterial growth from the inoculum-soil disks was suppressed either by acidifying the agar to pH 4 with phosphoric acid or by incorporation of streptomycin at pH 6.5. The number of pure colonies of the inoculant fungus from a total of twenty disks for each mixture was later recorded. Partial colonies, i.e. a sector of the inoculant fungus in a composite colony with one or more other fungi, were recorded as fractions.

Results of both studies have been summarized by Wastie (1961), who confined his analysis to the two fungal characteristics that seemed likely to

determine competitive ability on this substrate (1) growth rate of the inoculant fungus on virgin agar of the same composition (2) its tolerance of fungistatic growth-products excreted by other fungi on the same agar. To test this tolerance of fungistats, Wastie tested the growth rate of his various fungi over a circular sheet of Cellophane overlying standard agar plates that had been inoculated with soil 48 h previously. By comparing these figures with growth rates of the same fungi over Cellophane laid over virgin agar (not inoculated with soil), he was able to measure the tolerance of different fungal species to fungistats produced by fungi developing from soil inocula.

Success in competitive colonization of the nutrient agar by each of the tested fungi was expressed as percentage of sites on which a colony of the inoculant fungus arose, over a range of five inoculum-soil mixtures: 98, 90, 75, 50 and 10% inoculum. Analyzing the combined data from all his experiments, Wastie found a correlation between mean percentage colonization of the agar by each fungus and radial growth rate of fungal colonies on virgin agar in pure culture. Calculating a correlation coefficient for this possible association between mean percentage colonization (arcsin-transformed) and growth rate in pure culture, we find that $r = 0.6493$, which is significant at the 5% level. The relationship between mean percentage colonization and growth rate for eleven species of fungi for which full data were available is shown in Fig. 10.

No significant correlation was found to exist between mean percentage colonization by these eleven fungi and their tolerance of fungistats produced on this agar by other fungi from soil inocula. So these results have led to a simple conclusion about primary sugar fungi colonizing a virgin sugar substrate: they are successful pioneer colonizers because they get there first.

Wheat-straw units as the substrate

This was the first version of the Cambridge method, as pioneered by F. C. Butler (1953*a*) and R. L. Lucas (1955). The wheat-straw units used as a substrate for colonization were cut from long straw, each *ca.* 3.8 cm long × 4 mm external diameter, with a node at the lower end subtending a length of internodal culm with its enclosing leaf-sheath. In some experiments, straws were soaked in nutrient solutions of glucose and/or

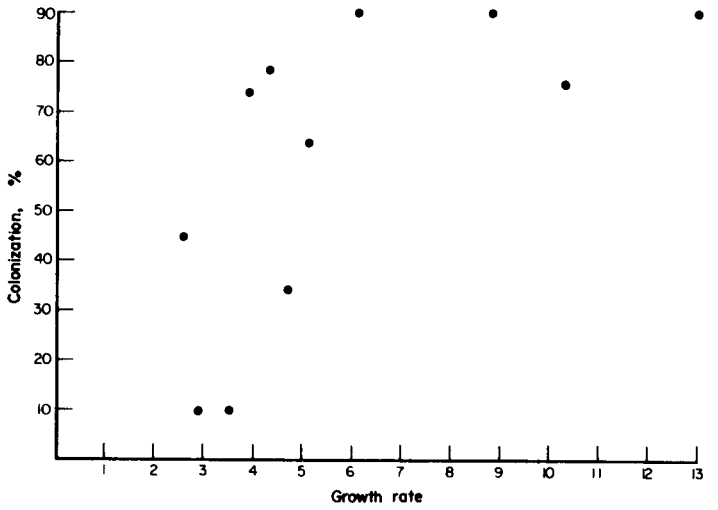

FIG. 10. Correlation diagram for eleven fungal species, showing association between fungal growth rate (mm/24h) over virgin nutrient agar and mean percentage colonization (arcsin-transformed) of similar agar from a range of inoculum-soil mixtures. Plotted from data given by R. L. Wastie (1961) in his Tables 1, 4, 5 and 6.

nitrate before sterilization by autoclaving, but we shall consider here only straws soaked in water alone. The autoclaved straws were buried in lots of 50 in cylindrical glass jars holding 200 ml of the various inoculum-soil mixtures and two jars (together holding 100 straws) were provided for each mixture of each fungus tested. After filling, jars were incubated for *ca.* 4 weeks at a laboratory temperature of 18–20°C, before determining percentage straws colonized by the inoculant fungus in each series. Four cereal foot-rot fungi, details of which are given in Chapter 8, were thus tested by Butler (1953*a*): *Fusarium culmorum*, *Curvularia ramosa*, *Helminthosporium sativum* (perfect state = *Cochliobolus sativus*) and *Gaeumannomyces graminis* var. *tritici* (formerly known as *Ophiobolus graminis*).

For each of these fungi, a reliable method had to be found for determining how many of the 100 straws had been colonized by the inoculant fungus, and so Butler tested and compared several methods. Plating-out of straws (after surface sterilization) on a nutrient agar was satisfactory only for *F. culmorum*, which is a fast-growing fungus (11 mm/24 h at 22.5°C) and so a successful colonizer of nutrient agar from a

mixed inoculum (see preceding section). *H. sativum* and *C. ramosa* produce large and easily recognizable conidia when incubated in a moist chamber and so Butler incubated the washed straws over a layer of moist sand in Petri dishes for 15 days at 25°C, rather longer than the minimum of 9 days since found adequate. A conidium of *H. sativum* is shown in Fig. 5. *G. graminis* does not readily produce recognizable conidia in culture and so Butler employed a method that I had devised for work on saprophytic survival of this fungus in similar wheat-straw units (Garrett, 1938; see Ch. 8). After straws had been washed, each one was split slightly at the top end and a wheat seed inserted in the lumen. The inseminated straws were then planted in boxes of moist sand; after 3 weeks growth in the glasshouse, wheat seedlings were washed out of the sand, and the roots of each one were examined for the dark-coloured runner hyphae of *G. graminis* (see Fig. 16). Four infected seedlings, together with four healthy ones, are shown in Plate 2.

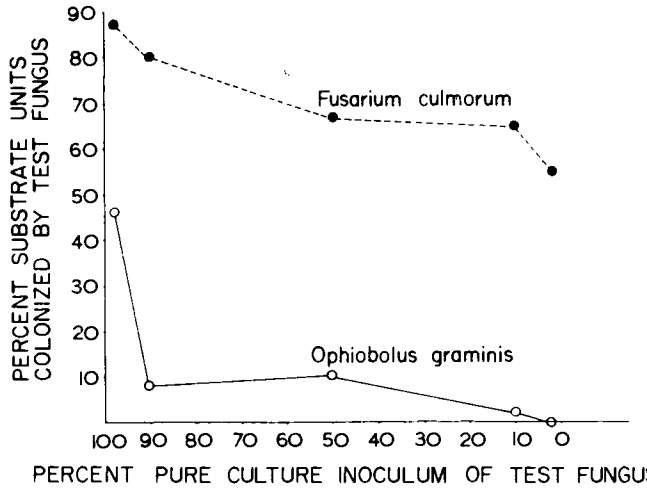

FIG. 11. Percentage saprophytic colonization of substrate units by *Ophiobolus graminis* compared with that by *Fusarium culmorum* over a wide range of inoculum potential (Drawn from data published by F. C. Butler, 1953a).

Typical results for two of these fungi, *Fusarium culmorum* and *Ophiobolus graminis* (labelled under its former name in Fig. 11), are shown in

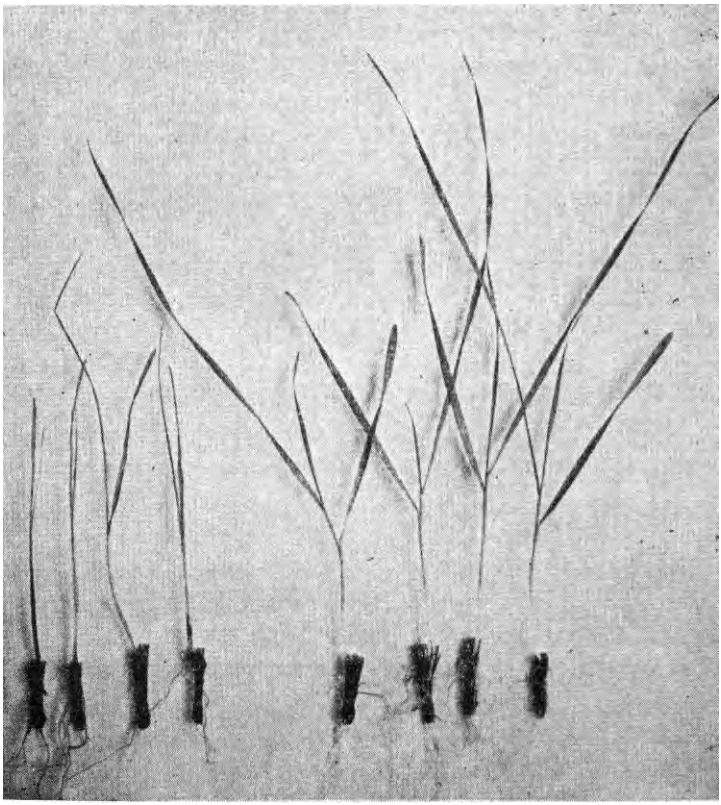

PLATE 2. Wheat seedling test for the detection of *Ophiobolus graminis* in pieces of wheat straw. The four stunted and sickly wheat seedlings on the left are encased by straw containing active *O. graminis*; the fungus is not present in the four straws on the right, so that the wheat seedlings are vigorous and healthy (From S. D. Garrett (1938). By courtesy of the *Annals of Applied Biology*. Copyright, Rothamsted Experimental Station).

Fig. 11, drawn from data in Butler's (1953*a*) paper. For each fungus, percentage of straws colonized declined with proportion of inoculum in the inoculum-soil mixtures. But the graphs for these two fungi are very unlike and show that *F. culmorum* is a vigorous competitive colonizer of wheat straw, whereas *O. graminis* is a poor one. This conclusion agreed with early work by T. S. Sadasivan (1939) on *F. culmorum* as a vigorous colonizer of wheat straw from natural inoculum present in cultivated soils at the Rothamsted Experimental Station.

The next objective was to determine those characteristics of a fungal species that conferred on it a high degree of competitive saprophytic ability for colonization of wheat straw. Here an important advance was made by R. C. F. Macer (1961*a*). Employing the four species studied by Butler (1953*a*), together with *Pseudocercosporella herpotrichoides* (formerly known as *Cercosporella herpotrichoides*; see Ch. 8), Macer determined the time taken by each fungus to penetrate wheat straws, from the outside of the enclosing leaf-sheath to the inside of the lumen; his test was carried out under axenic conditions with straws buried in a pure culture of each fungus. From his data, Macer concluded that straw-penetration rate of a fungus was more relevant to its degree of success in competitive colonization than was its radial growth rate over nutrient agar.

Later on (Garrett, 1975), I made a further advance in interpretation of the collected data, by using my own data on cellulolytic ability of these five fungi, which were not obtained until after Macer had completed his work. My work on cellulolytic ability will be described in Chapter 8, so it will suffice here to say that the method estimated percentage loss of dry weight by cultures of a fungus on filter-paper cellulose incubated for 7 weeks at 22.5°C. I found that my figures for cellulolytic ability of these five fungal species were correlated with Macer's figures for their respective rates of straw penetration; the correlation coefficient (r) was 0.9741, significant for five sets of variables at the 0.1 % level. I next summarized all comparable data on competitive colonization of wheat straw by these five species. The colonization rating for each species could be expressed by a single figure, the C_{50} *value*; this represents the highest percentage of unsterilized soil in the inoculum-soil mixtures that still permits the inoculant fungus to colonize 50 % of the straws tested. So the higher the C_{50} value, the greater is the competitive saprophytic ability of a fungal species for straw colonization. Calculation of the correlation coefficients showed that

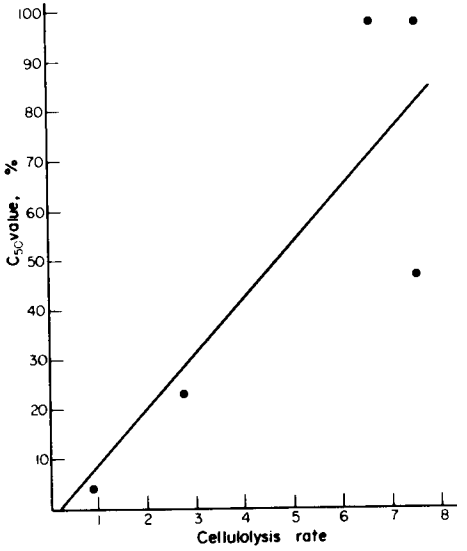

FIG. 12. Regression of C50 value for straw colonization on cellulolysis rate for five species of cereal foot-rot fungi. Plotted from data assembled by S. D. Garrett (1975).

$r = 0.8651$ for correlation between C_{50} value and straw-penetration rate, and that $r = 0.8378$ for correlation between C_{50} value and cellulolysis rate. Figure 12 shows the relationship between C_{50} value and cellulolysis rate for these five fungal species.

Figure 12 suggests that cellulolysis rate is the chief determinant of success in competitive colonization (represented by the C_{50} value). This is not surprising, because cellulolysis rate controls straw-penetration rate; cell walls in the straw tissue are dissolved by cellulase enzymes, produced by young penetrating hyphae, and penetration is hastened by exertion of growth pressure by the hyphal apices. So we can finally discern a parallel between saprophytic ability for competitive colonization of nutrient agar and of wheat-straw tissue, respectively. In each case, success depends on the speed with which a fungus can colonize and occupy a virgin substrate ahead of its competitors.

CHAPTER 8

SAPROPHYTIC SURVIVAL BY SOIL FUNGI ON COLONIZED SUBSTRATES

In the preceding chapter we discussed factors affecting competitive saprophytic colonization of substrates by soil fungi; in this chapter we shall consider how fungi exploit their colonized substrates for survival until a fresh substrate becomes locally available. Survival of fungi on substrates occurs in two ways:

(1) By continued saprophytic exploitation of the substrate. In the residues of annual or biennial plants, primary fungal colonizers may remain alive for one to several years, depending on the fungal species, the type of soil, soil conditions and depth of burial in the soil. In the woody tissues of tree roots, collar or trunk, the primary colonizer may sometimes remain alive for 10 years or more, depending on the bulk (diameter) of woody tissue.

(2) By dormant survival in the form of sexually produced oospores or ascospores, asexually produced chlamydospores, undifferentiated dormant mycelium or multicellular resting bodies known as sclerotia. The term "dormancy" of course implies inactivity but its most important characteristic for survival is a minimal respiration rate, as in dormant seeds of flowering plants; the consequence of this is conservation of carbon (energy) reserves and hence maximum longevity of survival in the dormant condition, until germination is triggered by a nutrient or other stimulus from a potential substrate.

These two methods of survival are not mutually exclusive. Some soil fungi produce no resting bodies and survive by continued though slow saprophytic activity within the colonized substrate. Others consume as

much of the substrate as they are able to decompose and finally produce large numbers of resting bodies from the reserve nutrients accumulated in their mycelium. Exhaustion of the available nitrogen supply is probably the commonest cause of cessation of mycelial activity and hence of resting-body production. For the understanding of fungal survival on colonized substrates, autecological studies have been essential; they are necessarily both exhaustive and prolonged, and so have been confined to fungal species of great economic importance. Such species include the cultivated mushroom (*Agaricus bisporus*) and various other species used for production of edible fruit-bodies by cultivation on composts, particularly in the tropics. But production of mushrooms and other toadstools in this way is a short-cycle industrial process; fresh inoculum is produced for each batch of compost and so the practical problem of fungal survival does not arise. For plant pathologists, however, a problem of great practical importance is posed by the survival of pathogenic root-infecting fungi in the residues left behind in the soil after harvest of a diseased crop. This problem has been more thoroughly investigated for a group of cereal root- and foot-rot fungal pathogens than for any other fungal group. So the remainder of this chapter will be devoted to a discussion of this particular study, in the hope that it will serve to light the way for further studies of the same kind but with other fungal groups.

FUNGAL SURVIVAL IN INFECTED OR SAPROPHYTICALLY COLONIZED WHEAT-STRAW TISSUES

The work now to be described has obvious parallels with the work on competitive saprophytic colonization of wheat straw described in the preceding chapter, though the problem to be investigated has been quite a different one. Thus the wheat-straw units used in the Cambridge method were similar to those that I had devised for my first study (Garrett, 1938) of saprophytic survival by the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*, to which I shall hereafter refer simply as *G. graminis*. Similarly, the wheat-seedling test for detection of living *G. graminis* in the straw units that I had devised for this early work on survival was again employed both for this fungus and for *Fusarium culmorum* in the Cambridge method; a modification of it was developed by Macer (1961*a*) for testing for presence of viable mycelium of the cereal eye-spot fungus

(*Pseudocercosporella herpotrichoides*) in straw units employed either for the Cambridge method (Macer, 1961*a*) or for studies of saprophytic survival (Macer, 1961*b*).

A brief note on the six fungal species involved in this study, and of their behaviour as parasites of wheat and other cereals, will be helpful here. The most specialized and dangerous pathogen is *G. graminis* var. *tritici*, which causes take-all and whiteheads in wheat and barley crops; var. *avenae* can attack oat crops as well. The fungus infects the cereal root-system from inocula lying in the soil and by ectotrophic growth along the roots eventually reaches the crown, consisting of the attached tiller-bases with their crown roots. *F. culmorum*, *Curvularia ramosa* and *Cochliobolus sativus* form a group of unspecialized pathogens infecting both young roots and senescent roots, though they lack the ability for continuous, ectotrophic spread over the cereal root system that is possessed by *G. graminis* (Fig. 16, Ch. 9). They can cause seedling blight of cereals, but infection of older plants, which may be directly through the crown tissue from the surrounding soil, appears to be restricted to certain soil and environmental conditions that predispose the cereal plants to such infections. *Pseudocercosporella herpotrichoides* is a more highly specialized pathogen, akin to *G. graminis*; it cannot infect roots or other underground organs but causes eyespot lesions of the tiller-bases above soil level, through infection by rain-splashed spores. Infected tiller-bases may be so weakened by the developing eyespot lesions that they cause the tillers to buckle above soil level at heading time; if the proportion of such straggled tillers is high enough in any area of the crop, then that area will fall down, or "lodge", as harvest time approaches, with a sometimes severe loss of grain yield. The sixth fungal species to have been included in these studies of survival is not a pathogen at all; *Phialophora graminicola* is a harmless parasite of cereal roots and of much interest because it exerts a valuable degree of natural biological control of the take-all disease (Ch. 9).

After harvest of a cereal crop in which one or more of these five fungal pathogens has caused an appreciable amount of disease, the infected stubble left behind by the combine harvester will cause a disease risk to any susceptible cereal that is sown within a year of harvesting the diseased crop; for the eyespot disease one year's break, which is usually sufficient for control of take-all, will not be long enough. The heat generated by burning the field of stubble may reduce infectivity somewhat but is quite inadequate

to destroy it completely. So the main research problem has been to determine precisely how soil conditions affect the survival of each of these five fungal pathogens. Once the mechanism of survival can be thoroughly understood, then it is often possible to devise appropriate measures of crop husbandry whereby the farmer can shorten pathogen survival to an acceptable limit. This kind of problem is entirely suitable for laboratory experimentation, though for wheat-seedling testing for survival of some of these fungi it is convenient to have glasshouse facilities. Glass jars (300 ml) have usually been used to hold *ca.* 200 g (air-dry) soil, and in each of these are buried fifty straw-units that have been colonized by the fungus in axenic (i.e. pure) culture for an incubation period of *ca.* 1 month. Straw-units (hereafter referred to as "straws") prepared in this way can be made available in any desired quantity at any time throughout the year and so are more convenient for the purpose than the collection of naturally infected stubble from diseased fields around harvest time.

It is advisable to check any laboratory technique against conditions outside in the field and so I will now describe such a check that I carried out at the Rothamsted Experimental Station during my work on saprophytic survival of *G. graminis* (Garrett, 1940). The three comparisons tested in this experiment were (1) between straws artificially colonized by *G. graminis* in axenic culture *v* similar lengths of naturally infected tiller-bases collected from a diseased crop (2) between a Rothamsted clay-loam soil and a light-textured sandy loam from Bridgham in the county of Norfolk (3) between straws buried in soil jars, incubated in the laboratory, and others buried in small plots of the two soils outside in the field. The experiment was designed as a factorial arrangement of $2 \times 2 \times 2$ treatments, but was incomplete because the number of naturally infected straws available was insufficient (Table 5). The results of such an experiment can be presented in either tabular or graphical form, showing the gradual decline in viability of the fungus in the various treatment-series over the whole period of sampling. A more recent method (Garrett, 1976), which is both more economical and more precise, is to calculate for each treatment-series the median survival-period of the fungus, i.e. the period for which it has survived in 50 % of straws sampled (the S50 value). This is done by calculating the regression of percent survival (y) on time (x). By giving y a value of 50 in the regression equation connecting x and y, the S50 value can quickly be calculated (Bishop, 1966, Ch. 7). This method of calculating the regression of percent

survival on time can be legitimately employed only if the successive survival-points on a graph fall approximately along a descending straight line (see Fig. 13). For all the survival-data I have thus surveyed (Garrett, 1976), such a straight-line relationship has nearly always been obtained, and the rare departures from it can usually be attributed to experimental error. For the experiment described above, S50 values for *G. graminis* in the six treatment-series are set out in Table 5.

TABLE 5. MEDIAN SURVIVAL PERIODS OF G. GRAMINIS IN SOIL

	S50 period (months)	
	Rothamsted soil	Bridgham soil
Artificially colonized straws		
Laboratory containers	1.25	3.56
Field plots	6.03	8.41
Naturally infected straws		
Field plots	5.96	6.44

Table 5 shows that artificially colonized straws when buried in field plots gave an S50 period in Rothamsted soil almost identical with that for naturally infected straws. In Bridgham soil, however, the naturally infected straws gave an S50 period only three-quarters of that given by artificially colonized straws. This result suggests that artificial colonization of the straws is more uniform and complete than is natural infection of living plant tissues, and so it justified the continued use of pure-culture straws for later experiments. The second important feature of Table 5 is the demonstration that S50 periods for *G. graminis* in colonized straws are much shorter in laboratory soil containers than outside in field plots. This is not surprising because mean temperature in the laboratory is much higher than soil temperature outside, except during the warm months of summer. Exhaustion of a fungal substrate is certainly a biological activity and so it is accelerated by rise in temperature, at least up to 25°C. This comparison shows the saving of time afforded by laboratory experimentation, which also permits a closer control of soil conditions, such as moisture content, temperature and nutrient status, than is possible in the field or glasshouse.

We should expect that *G. graminis*, or any other primary fungal colonizer of mature plant tissue, will exhaust its substrate and so die out most quickly

under conditions optimum for soil microbial activity, i.e. under much those conditions optimum for infection of living host tissues in the first place. These expectations have been confirmed by experimental work in almost every particular (Garrett, 1981). Thus *G. graminis* dies out most quickly in warm, moist and loose (i.e. well aerated soils); conversely, it survives for longest in cold or in air-dry soils, in which it is preserved in cold or dry storage respectively. So its viability is little reduced during the cold European and N. American winters, and during the long dry summer in South Australia, for example. But in another Australian State, Queensland, regular summer rainfall occurs, producing a warm, moist condition of the soil. MacNish (1973) has suggested that such a soil condition, if sufficiently prolonged, will shorten saprophytic survival of *G. graminis*; this may indeed be the reason why take-all is not a problem in Queensland wheat crops, even though *G. graminis* occurs in the soils.

Effects of soluble soil nitrogen on saprophytic survival

There is one outstanding exception to the above generalization that *G. graminis* dies out most quickly from its straw substrate under conditions conducive to maximum microbial activity. For maximum longevity of survival, this fungus needs a maintained supply of soluble nitrogen, normally in the form of nitrate, diffusing into the straw tissue from the surrounding soil. In many experimental trials, the optimum level of nitrate corresponded to *ca.* 0.5 gN/100 g air-dry straw. Figure 13 shows the time-course of saprophytic survival by *G. graminis* in colonized straws buried in a Rothamsted clay-loam soil; the two treatments were (1) addition of calcium nitrate at the rate of 0.45 gN/100 g straw (2) no addition (Garrett, 1944, Table 1). From the survival-data for these two treatment-series, the regression of percent-survival on time (in weeks) was calculated for each series and the regression lines are shown in Fig. 13.

Figure 13 is representative of regressions of percent-survival on time drawn from more than a dozen collections of survival data for *G. graminis* (Garrett, 1976); it shows that the survival points for both treatment-series fall closely along the regression lines. The effect of calcium nitrate in prolonging longevity of the fungus is striking, and similar effects were produced by addition of either ammonium nitrate or ammonium sulphate at the same rate. Because cellulase enzymes are produced only by *young*

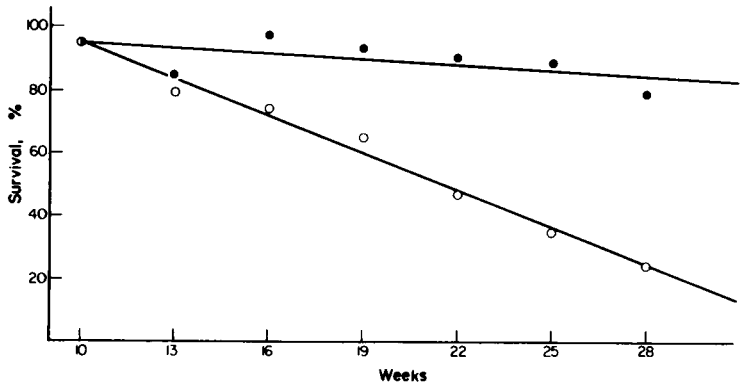

FIG. 13. Survival of *Gaeumannomyces graminis* var. *tritici* in colonized wheat straws buried in a Rothamsted arable soil. Open symbols: no nitrogen added to soil. Closed symbols: calcium nitrate added at rate of 0.45g N/100g air-dry straw. Plotted from data in Table 1 of S. D. Garrett (1944).

hyphae of the fungus, prolonged saprophytic survival requires a slow but continued production of young branch hyphae (for which a supply of nitrogen and other mineral nutrients is needed); these continue to degrade the cellulosic substrate, producing by enzymic hydrolysis the sugars needed to support respiration and the slow but continued development of the fungal colony within the straw tissue. I was able to demonstrate this by growing axenic cultures of *G. graminis* on single circles (7 cm diam.) of Whatman no. 3 filter-paper (mean air-dry weight = 0.71 g) standing on a bed of acid-washed sand, holding a sufficient reserve of mineral nutrient solution (Garrett, 1967). Percent-survival of the fungus at different levels of sodium nitrate after 23½ weeks at 22.5°C was estimated by a wheat-seedling test (Fig. 14).

Figure 14 shows that the level of sodium nitrate promoting maximum longevity of *G. graminis* was 0.33 g/litre. Below this level of nitrate, the fungus died out prematurely from carbohydrate starvation, because lack of nitrogen prevented it from continuing to replenish its mycelium, and so it was unable to hydrolyse the remaining cellulose. At a level of nitrate above the optimum, the fungus hydrolysed cellulose at a rate higher than that required to maintain the colony, and so the supply of cellulose was prematurely exhausted. Similar but more delayed effects of both kinds were

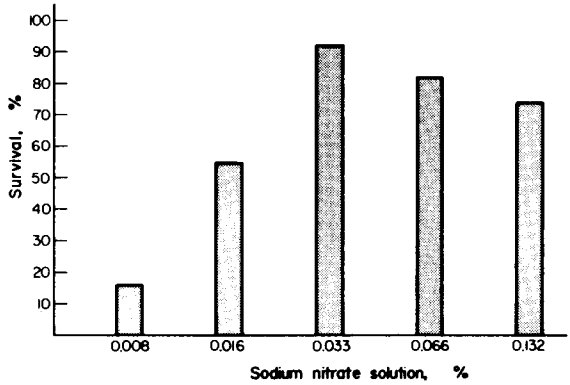

FIG. 14. Survival of *Gaeumannomyces graminis* var. *tritici* in axenic culture on filter-paper cellulose, at five levels of sodium nitrate in solution. Data from Table 3 in S. D. Garrett (1967).

later demonstrated with *Phialophora graminicola* similarly growing in axenic culture on thinner circles (Whatman no. 2) of filter-paper (Garrett, 1971, Table 1). But for maximum longevity of *G. graminis* in colonized straws buried in soil, the optimum level of sodium nitrate is about 10 × that required for maximum longevity on filter-paper in axenic culture, i.e. in a nitrogen-poor clay-loam soil maintained at a moisture content of 50% saturation, the required concentration of sodium nitrate works out at *ca.* 3.3 g/litre. This much higher N-requirement in the soil can be ascribed to competition with other soil micro-organisms, which soon invade the colonized straws after burial and take up nitrate from the surrounding soil.

Although other micro-organisms thus compete with *G. graminis* for both carbon constituents of the straw tissue and nitrogen, yet the most striking fact about the saprophytic survival of this fungus is its truly remarkable tolerance of microbial competition during survival. In a nitrogen-rich soil, it continues to survive even when the straws have reached so advanced a stage of decomposition that they are difficult to handle for the wheat-seedling test. Data provided by Butler (1959) show that *Fusarium culmorum* responded to nitrogen in its longevity of survival in straws just as strongly as does *G. graminis*. Its tolerance of microbial competition during survival was equally great; after 72 weeks in Butler's nitrogen-rich soil, individual straws had become so decomposed that they broke up during attempted

recovery from the soil. Nevertheless, Butler planted wheat seedlings in the mixture of straw fragments and soil; after 72 weeks, 35% of the test seedlings were infected and after 104 weeks 37% infected seedlings were recorded. This result for *F. culmorum* was not difficult to understand because, as Fig. 11 shows, it is a vigorous competitive colonizer of fresh wheat straw in tests by the Cambridge method. Figure 11 also shows, however, that *G. graminis* is a poor colonizer of fresh straw when tested by this technique, and so its great tolerance of microbial competition during survival is more difficult to explain. Nevertheless, we can understand that competition for survival on a substrate is likely to be less severe than competition for colonization of the substrate in the first place. During natural infection of wheat tiller-bases in the field, a primary pathogen like *G. graminis* is likely to occupy most of the host tissue, because host resistance at first excludes following, secondary micro-organisms. Similarly, autoclaved straws inoculated with a pure culture of *G. graminis* are completely colonized by it when the incubation period has been completed. Complete occupation of a plant-tissue substrate by either *G. graminis* or *F. culmorum* gives the primary fungal colonizer a great initial advantage that it is able to maintain against secondary invaders even under the intensified microbial competition that is caused by a continued supply of nitrate-N. But not all fungal species in this group are so tolerant of the intensified microbial competition characteristic of nitrogen-rich soils, as we shall see in the next section.

Tolerance of microbial competition and other factors affecting saprophytic survival

In contrast to the behaviour of *G. graminis* and *F. culmorum*, two other species in this group are intolerant of the intensified microbial competition induced by nitrate supplementation of the soil; an excess of soluble nitrogen does not prolong saprophytic survival but sharply reduces it. This has been repeatedly demonstrated for various isolates of *Cochlibolus sativus* (Butler, 1953*b*; 1959; Garrett, 1966; 1978). A similar result for *Phialophora graminicola* was reported by Balis (1970), who found that reduction in longevity of *P. graminicola* increased with concentration of nitrate up to a level of 12.5 mgN/100 g air-dry soil, corresponding in this experiment to a dose of 0.6gN/100 g air-dry straw.

The difference in nitrogen-response between these two pairs of fungi is not difficult to understand or even to explain. But when I add that the remaining two fungi, i.e. *Pseudocercosporella herpotrichoides* and *Curvularia ramosa*, show an indifferent response to nitrate-N, because it does not significantly affect their longevity of survival, then the complexity of the situation becomes apparent. Nevertheless, it is possible to explain all three types of nitrogen response—positive, negative and indifferent—on the basis of three intrinsic characteristics of each fungal species; some variation between different isolates of a fungal species in respect of these characteristics naturally occurs, but does not affect the general explanation. These three characteristics are:

(1) *Cellulolysis rate.* This determines the rate at which sugars are liberated by hydrolysis of cellulose and has been estimated by percent-loss in dry weight of filter-paper cultures incubated for 7 weeks at 22.5°C (Garrett, 1966) or by some variant of this general method (Forbes and Dickinson, 1977; Deacon, 1979). The most direct evidence that the S50 period of a fungal isolate is partly determined by its cellulolysis rate comes from my own study of five isolates of *Cochliobolus sativus*; there was a positive correlation, significant at the 1% level, between cellulolysis rate and S50 period among the five isolates (Garrett, 1978).

(2) *General metabolic rate.* Fungal growth rate over potato-dextrose agar at 22.5°C, expressed as mm/24h, was originally chosen as a quickly and easily determined parameter of general metabolic rate for this group of fungi (Garrett, 1966). Further evidence in support of this choice has recently been provided (Garrett, 1980).

(3) *Tolerance of microbial competition.* Independent evidence for variation in tolerance amongst these six fungal species has been provided, partly by the Cambridge method and partly by various kinds of test on nutrient agar plates. Thus *Fusarium culmorum* and *Curvularia ramosa* were much more successful in competitive colonization of wheat straw by the Cambridge method than were the other three species thus tested. In a comparison between *Curvularia ramosa* and *Cochliobolus sativus*, Butler (1953*a*) found that *C. ramosa* was much more tolerant of anti-fungal antibiotics and of bacterial interference on nutrient agar plates than was *C. sativus*. Wastie (1961, Table 2), by the use of disks of Cellophane film laid over a plate of nutrient agar, devised a technique for assessing the tolerance of root-infecting fungi to fungistatic products formed by micro-organisms

growing from a soil inoculum. Values of Wastie's reduction factor for the four species cited above were as follows: *F. culmorum*, 0.87; *C. ramosa*, 0.51; *G. graminis*, 0.24; *C. sativus*, 0.16. As we have noted in the discussion of Wastie's work in Ch. 7, conditions of microbial competition on the agar plate, with its high concentration of nutrients, are different from those during colonization of wheat straw by the Cambridge method. Again, as I have remarked earlier in the present chapter, *G. graminis* is far more tolerant of microbial competition during its saprophytic survival in buried wheat straws than would have been forecast from its poor performance in trials either of the original Cambridge method or of its agar-plate modification. So this is one more example of a lesson now well learnt by soil microbiologists, i.e. that results from agar-plate techniques can be extrapolated to the soil environment only with extreme caution.

From the evidence so far produced, we can draw three conclusions. The first is that the S50 period may be directly correlated with cellulolysis rate. The second is that low cellulolysis rate can be compensated by a continued supply of soluble nitrogen to the straw from the surrounding soil; this enables cellulolysis by the fungal colony to continue when a lack of available nitrogen would have brought it to a halt. The third conclusion is that an unlimited supply of soluble nitrogen will benefit survival only if the primary fungal colonizer is sufficiently tolerant of the intensified microbial competition that occurs in a nitrogen-rich soil; if it is not, then additional nitrogen will produce the reverse effect of shortening survival. We can now look at the tabulated data on cellulolysis rates and nitrogen responses in saprophytic survival for all six fungal species and see how far these three conclusions will take us (Table 6).

We can look at Table 6 firstly to see whether a low cellulolysis rate (as expressed by mean loss in paper dry wt, 1st column) is associated with a positive response to nitrogen and a high rate with an indifferent or negative response. We find two exceptions to this expectation; *F. culmorum*, with the highest cellulolysis rate (7.5%), shows a positive response, and *P. herpotrichoides*, with the lowest rate (0.9%) shows an indifferent instead of a positive response.

Further research has provided explanations for both these exceptions to expectation, and we can deal with *P. herpotrichoides* first, because it appears to be a special case. Deacon (1973*a*) has studied both cellulolysis rate and survival in straws of five isolates of this fungus and has suggested

TABLE 6. CELLULOLYSIS RATES, CAI VALUES AND NITROGEN RESPONSES IN SAPROPHYTIC SURVIVAL

	Mean % loss in filter-paper dry wt. after 7 wks at 22.5°C	Linear growth rate of fungus (mm/24 h) at 22.5°C	CAI value	Nitrogen response
G. graminis (5 isolates)	2.8	7.3	0.38	Positive
F. culmorum (4 isolates)	7.5	11.0	0.68	Positive
P. herpotrichoides (4 isolates)	0.9	1.3	0.69	Indifferent
C. ramosa (1 isolate)	6.6	5.3	1.25	Indifferent
P. graminicola (4 isolates)	5.3	3.0	1.77	Negative
C. sativus (6 isolates)	7.5	3.6	2.08	Negative

that cellulolysis rates are too low to maintain active saprophytic survival. He has concluded that the fungus survives as well nourished dormant mycelium, though it is not morphologically differentiated in any microscopically obvious way. Dormancy can explain its indifferent response to nitrogen in survival, and is compatible with the exceptionally long period of survival in colonized straws buried in field plots that was reported by Macer (1961*b*, Tables 3 and 4). After three years, the fungus was still alive in 76% of the straws buried at ploughing depth (15 cm); compare this with data for *G. graminis* in Table 5 above.

The difficulty posed by the behaviour of *F. culmorum* was a more fundamental one, so that when I was originally faced by this problem I had to reconsider the whole question of how cellulolysis rate is connected with the energy requirements of a fungus during its saprophytic survival in a cellulosic plant tissue (Garrett, 1966). I eventually realized that the energy needs of a fungus during saprophytic survival are determined by its general metabolic rate, i.e. a fast-growing fungus needs a higher rate of sugar liberation to supply mycelial respiration than does a slow-growing one. So in distinguishing between the behaviour of different fungal species, it is not enough to consider cellulolysis rate by itself; differences in fungal behaviour are more likely to be governed by cellulolysis rate in relation to

general metabolic rate, i.e. by the ratio between these two values. This ratio I then christened the *cellulolysis adequacy index*, or CAI value. The numerator and denominator of this ratio are given in the 1st and 2nd columns, respectively, of Table 6 and the resulting CAI value in the 3rd column.

We can now examine from Table 6 the relation between CAI value and nitrogen response in survival. If we accept Deacon's (1973*a*) conclusion that *P. herpotrichoides* survives by dormant and not by active saprophytic mycelium, then we can exclude this species from further consideration, and concentrate on the remaining five. We find that for two species, *G. graminis* and *F. culmorum*, the CAI value is significantly below unity and is associated with a positive response to nitrogen in survival. In the remaining three species CAI values are significantly above unity and are associated with either an indifferent (*C. ramosa*) or a negative (*P. graminicola* and *C. sativus*) response to nitrogen. The factor that determines whether the nitrogen response is indifferent or negative must be the degree of tolerance to microbial competition during saprophytic survival. Thus, *C. ramosa*, with an indifferent response, is highly tolerant, whereas *C. sativus*, with a negative response, is intolerant.

Wider applications of the cellulolysis adequacy index

Deacon (1979) has recently made some valuable proposals for an extension of the CAI ratio to a wider range of soil fungi. His study began with an inquiry into cellulolytic ability in a range of eighteen species of *Pythium*; two methods were employed (1) loss of tensile strength by inoculated cellulose film with time, estimated by a weight-loaded 'penetrometer' (2) loss of dry weight by filter-paper cultures, by a method modified from the one that I had proposed in 1966. Agreement between results from these two methods was highly significant (at the 0.1 % level). Amongst these species, eight were classed as positively cellulolytic and the remainder were either non-cellulolytic or doubtful. The wider interests of this study emerged when Deacon, for his Table 3, calculated CAI values for four typical cellulose-decomposing species; percent losses in filter-paper dry weight covered the range 1.99–3.61, linear growth rates were 17–28 mm/24h, and CAI values 0.07–0.21. As shown by these figures for growth rate, *Pythium* species are typically fast-growing fungi; their high

growth rate has been ascribed by Trinci (1971) partly to their coenocytic mycelium, during extension growth of which there are no septa and hence no barriers to the streaming of protoplasm towards the growing hyphal apex. The CAI values for these four *Pythium* spp. are low, and suggest that cellulose is not biochemically their primary substrate. In his Table 3, Deacon has compared these data for his *Pythium* spp. with corresponding data for the cereal fungi considered above and some related fungi. Figures are also given for *Botryotrichum piluliferum*, a soil fungus that is a typical strong decomposer of cellulose; cellulolysis rate was 12.4%, growth rate was 2.5 mm and CAI value was thus 4.96. These figures are probably fairly typical for soil fungi that are strong decomposers of cellulose. Those *Pythium* species that can decompose cellulose often represent the opposite extreme, i.e. only a moderate cellulolysis rate allied with a high growth rate. Species of *Pythium* were formerly thought to be incapable of cellulose decomposition, and were hence termed "sugar fungi" (Ch. 7).

So Deacon has argued, quite correctly, that the term "sugar fungi" is no longer appropriate for those *Pythium* species that have been shown to be capable of decomposing cellulose. Nevertheless, difficulties of terminology do not necessarily invalidate the general concept of such a group of pioneer colonizers, for which fresh plant tissues are the usual substrate, because "unfresh" tissues are often already colonized. Similar difficulties of terminology also beset use of the term "saprophytic" in the phrase "saprophytic sugar fungi", because fresh plant tissues are usually alive when they fall upon or are incorporated with the soil; severance of a corpus of fresh plant tissue from its parent plant does not thereby kill the tissue, which remains alive for some time, even though its resistance to microbial invasion may be sharply reduced by severance.

So I have to admit that some members of this natural ecological group of fungi that are pioneer colonizers of virgin plant tissues, usually alive at the time of fungal invasion, have been wrongly named as "saprophytic sugar fungi"; some of them, as Deacon (1979) has shown, have at least a limited power of cellulose decomposition; usually such fungi are invading fresh plant tissues as parasites rather than as saprophytes. Such difficulties should not deter us from improving our attempts at ecological grouping as a guide to understanding the behaviour of soil fungi. The history of ecological classification reveals the frequent inability of the human mind,

which craves for clear-cut boundaries, to cope with the infinite continuum of variation in the natural world. In every generation many biologists have had to re-learn, in frustration and disappointment, the lesson taught by Charles Darwin in *The Origin of Species*.

CHAPTER 9

ROOT-INFECTING FUNGI

Root-infecting fungi are distinguished by their ability to feed on living plant tissues, for which they must be able either to overcome or to evade host-plant resistance to invasion. All such fungi live naturally upon the roots, rhizomes and other underground organs of their wild host plants but pathogenic fungi do not usually cause severe damage to their wild hosts in a natural plant community. In such a community, host-parasite relationships tend to evolve towards a balanced state, for which less aggressive strains of the fungal parasite become selected by greater fitness for survival. Such a balance is advantageous to the fungal species, because the size of its populations and the extent of its geographical range ultimately depend on those of its hosts; this generalization applies, however, only to specialized parasites with a limited range of related host species. This is expressed by shepherds in the saying "Happy worms in happy sheep". This idealized situation is likely to be true for a flock of sheep living on semi-natural grassland under living conditions rather better than those for their wild ancestors, but it is rarely true for populations of an agricultural crop plant. Crops are grown in pure stands of a single cultivar of a single species; any specialized fungal parasite that can spread progressively over the roots of that crop is able to cause widespread damage. In wild vegetation, on the other hand, there is a mixture of plant species; a specialized parasite infecting the roots of a susceptible plant may be unable to spread further from that particular focus of infection, because it is separated from other susceptible plants by "root barriers", i.e. the root systems of surrounding plants that may happen to be resistant to infection by that particular specialized parasite.

Two sub-classes of root-infecting fungi can be distinguished:

(1) Pathogenic root-infecting fungi, causing disease.

(2) Mycorrhizal fungi, infection of rootlets by which produces a persistent, composite organ known as a *mycorrhiza*, in which root and fungus exist together in apparent harmony. Such a relationship is termed a *mutualistic symbiosis*, because both partners derive some benefit from the association. In my treatment of both these groups of fungi, I shall for convenience make one assumption, *viz.* that specialized parasites have evolved from unspecialized parasites, rather than vice versa, as has been argued by some microbiologists. The fossil record for fungi is scanty, because they leave behind no mineralized skeleton; available records have been summarized by K. A. Pirozynski (1976). Nevertheless, it is unwise to label unspecialized parasites as "primitive", because their abundance in soil and their breadth of geographical distribution usually exceed those of specialized parasites.

PATHOGENIC ROOT-INFECTING FUNGI

Unspecialized parasites

In these fungi, parasitism is usually limited by the resistance to fungal invasion of mature host tissues, so that infection is limited to juvenile tissues, or to older tissues of plants predisposed to infection by some severely adverse condition of their environment, such as a toxin or a crippling deficiency of some nutrient. Species of *Pythium* and *Rhizoctonia solani* are widespread parasites of this kind, affecting seedlings and young plants. But damage of this kind is not confined to young plants, because all plants go on producing juvenile tissues, in the form of root tips, throughout the growing season. If a large population of such a pathogen has developed under soil conditions favourable for its activity, then even large trees can be killed through a progressive destruction of root apices. A good example is the widespread destruction of native eucalypt forests in Australia by *Phytophthora cinnamomi* (Podger, 1975).

Such unspecialized, necrotrophic pathogens are limited by mature host-tissue resistance partly because their destructive invasion provokes an immediate and strong defence-response. Living plant tissues are invaded in

much the same way as a substrate of dead plant tissue is colonized, with a full deployment of tissue-degrading enzymes and sometimes of phytotoxins as well. So a high degree of competitive saprophytic ability (Ch. 7) is fully compatible with such a type of parasitism. *Rhizoctonia solani* is a successful colonizer of cellulosic substrates in soil, as well as a widespread parasite of young plants and a colonizer of senescent tissues. *Pythium* species are similarly quite omnivorous, though their cellulolytic ability is usually much lower than that of *R. solani* (Ch. 8). Nevertheless, they are the most frequent colonizers of living green tissues of weeds or catch crops ploughed under the soil.

Specialized parasites

There are two main types of *infection habit* amongst these fungi, apart from a small residue of miscellaneous types. In one group, the vascular-wilt pathogens, the fungus exploits the rather exclusive highway of the plant's vascular (conducting) system. In the other group, of ectotrophic parasites, the pathogen has evolved an infection habit whereby host resistance to invasion is continuously overcome, so that a progressive infection of the whole root system, and ultimately of the crown and shoot base(s), can develop unless checked by soil conditions sufficiently adverse to such fungal spread.

Vascular-wilt fungi

These fungi resemble unspecialized parasites in one respect; entry into the host plant is limited to juvenile tissues of young roots and rootlets. After invasion of the immature root cortex, the fungus passes through the yet incompletely suberized endodermis, enclosing the stele of young vascular tissue, and then into the vessels of the protoxylem. In a fully susceptible species of host plant, passage of the pathogen up the xylem vessels may proceed more or less uninterrupted by host defences, and the typical array of disease symptoms, collectively known as the *wilt syndrome*, eventually develops.

Nevertheless, different species of crop plant vary over a wide range in their degree of susceptibility to infection by these wilt pathogens, and in

many types of crop plant, breeders have produced cultivars that are resistant to disease development or tolerant of its effects. In such plants, the resistance of young root cortices has been considerably increased, and precocious suberzation of the endodermis may create an additional barrier to fungal invasion. So the impetus of the fungal invasion is reduced and fewer infections get through into the young xylem vessels. Their defence response is usually able to contain such a reduced number of fungal invasions; it is effected by a mechanism known as *tylosis*, which is characteristic of cells of the xylem parenchyma and is a general response to any kind of damage or injury to xylem tissue, i.e. it is not a response specific to invasion by fungal or bacterial pathogens. Xylem vessels are in contact with the parenchyma cells of medullary rays; over the area of such contact, the secondary wall of the vessel is perforated by circular pits, so that the vessel lumen is separated from the adjoining parenchyma cell only by the *pit membrane*, corresponding approximately to a thin primary wall. Any injury to the vessel, including an incipient infection, triggers off a reaction by the associated parenchyma cells; the pit membrane expands and grows into a balloon-like outgrowth intruding into the lumen of the vessel. Such an outgrowth is called a *tylose* (Fig. 15).

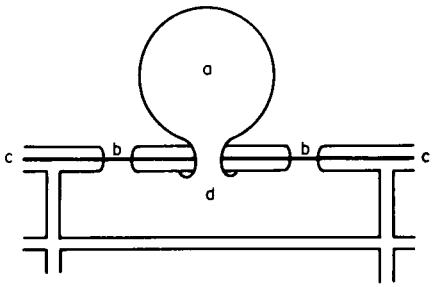

FIG. 15. Diagram of tylose development within vessel by outgrowth from adjoining ray-parenchyma cell: (a) tylose; (b) pit-closing membrane; (c) wall of vessel; (d) ray-parenchyma cell.

A number of such tyloses is usually produced in response either to injury or to vascular infection and this results in complete blocking of the vessel. Provided that tylosis of the xylem is not too extensive, this reaction does

not seriously affect upwards movement of the transpiration stream, especially as new secondary xylem is produced each season by the cambium. In hop plants (*Humulus lupulus*) with a mild infection by *Verticillium albo-atrum*, one of the symptoms is a conspicuous thickening of the stems due to enhanced production of secondary xylem, a symptom known to the growers as "thick bine". Monocotyledons have no mechanism for production of secondary xylem but appear to have surplus conducting capacity.

Vascular-wilt fungi appear unable to invade mature parenchyma cells, whether in the cortex, medullary rays or pith; in this limitation only they resemble unspecialized parasites. So a vessel completely occluded by tylosis appears to halt further advance of the pathogen up that particular file of vessels. This limitation has a further consequence; because the fungus is unable to traverse a barrier of mature parenchyma it is imprisoned within the vessels until the particular region of root or stem becomes precociously senescent as a result of infection. Then tissue resistance is sufficiently reduced for the fungus to break through the outer tissues of root and stem, and then to sporulate. This sequence of events, in tomato plants with Verticillium wilt, has been directly observed and photographed by G. W. F. Sewell (1959).

Once a progressive infection has been established in the vessels of the tap root (dicotyledons) or rhizome (monocotyledons), then subsequent upwards spread of the fungus is more rapid than in any other kind of fungal disease. This is because pathogens in the genera *Fusarium* and *Verticillium* produce large numbers of small conidia in the nutrient sap of xylem vessels, and thus are rapidly carried upwards in the transpiration stream. As a file of vessels matures, many of the end-walls between them are dissolved; end-walls that remain are quickly penetrated through the pit-membranes by germinating conidia, and the germ tubes bud off further conidia on the other side, into the next vessel. This sequence of events, in banana plants suffering from wilt due to *Fusarium oxysporum* f. *cubense*, has been observed by E. E. Trujillo (1963); he found that this fungus could migrate from a banana rhizome to the top of the pseudostem, 7.6 metres tall, in less than 14 days, thus moving at a rate of *ca.* 55 cm/24 h. References to other work on vascular-wilt diseases can be found in Chapter 3 of my *Pathogenic Root-infecting Fungi* (Garrett, 1970).

Ectotrophic root-infecting fungi

These fungi are thus named because of their epiphytic (over the plant surface) infection habit, which is shown for *Gaeumannomyces graminis* var. *tritici*, the fungus causing take-all of wheat and barley, in Fig. 16. The dark-coloured *runner hyphae* of this fungus grow over and along the root surface, sending hyaline (colourless) branches into the root cortex at short intervals. In due course, hyphae pass through the endodermis and into the vascular stele (not shown in Fig. 16b), which responds by a dark-brown discoloration of the vascular elements.

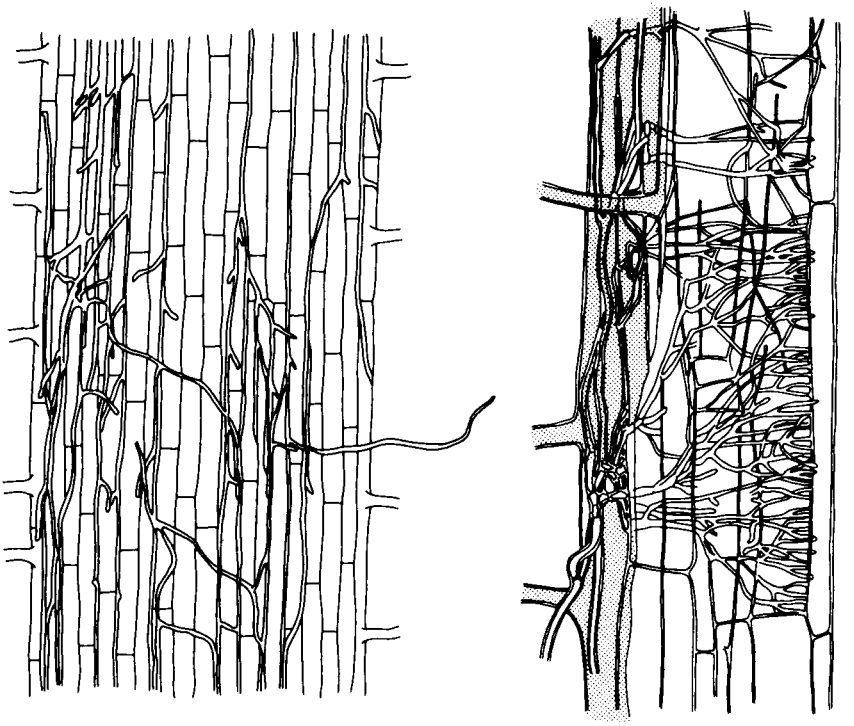

FIG. 16 Ectotrophic spread by *Ophiobolus graminis* along wheat root. *a*, surface view, showing dark-coloured runner hyphae; *b*, in longitudinal section, showing branch systems of colourless infection hyphae growing through the cortical cells of the root (After the drawing by G. Samuel, reproduced from S. D. Garrett (1934). By permission, *Journal of the Department of Agriculture of South Australia*).

If soil conditions are optimal for ectotrophic spread, i.e. in a well aerated, light-textured soil, neutral to slightly alkaline and at medium moisture content, then *G. graminis* spreads progressively over the root system. But if soil conditions are too unfavourable for surface growth over the roots, as in an acid soil at pH 4.0, then the fungus can spread only inside the root. There fungal growth is much slower, because it provokes a maximum defence-response of the host tissues, and this usually halts it completely. Just the same sequence of events occurs in leaf-spot diseases caused by fungal pathogens. Leaf spots are usually small and the size is typical for the particular disease, because development beyond this size is halted by host-defence reactions. Such reactions include production of phytoalexins and other fungistatic substances, advance lignification of host tissues ahead of fungal invasion, production of cork barriers, etc. But if the leaf is infected more or less simultaneously, under conditions of high humidity favourable for such infection, by a number of fungal spores, then a number of small leaf spots develops. If the density of such lesions exceeds a certain critical value, then host defences are saturated and can no longer keep the fungus in check; it breaks out of the primary lesions and begins to spread continuously through the leaf tissue. This is what happens when the *aggressive phase* of chocolate spot of field beans (*Vicia faba*) is caused by *Botrytis fabae*. The small leaf spots do little damage on their own, but in the aggressive phase *B. fabae* can destroy much of a bean crop if rainy and humid weather continues for long enough.

The sequence of events just described is termed *synergism between infections*; a sufficient number of infections *acting together* can overcome host resistance, whereas one infection by itself cannot do so. Such a synergism is achieved in another way by the ectotrophic infection-habit. When soil conditions permit, *G. graminis* grows from its food-base (usually another infected root or grass rhizome) over the root surface; from this travelling mycelium, a shortly consecutive series of invasions of the root cortex is initiated. Synergism between these infections suffices to overcome host resistance and infection proceeds as a continuous wave along the root. When I first described this infection habit for *G. graminis* (Garrett, 1934), I noted that when the fungus was growing downwards along a seedling root, it eventually abandoned the ectotrophic habit and the runner hyphae travelled intercellularly several cells deep in the cortex. This change I attributed to a decrease in host resistance in the distal part of the root,

virtually disconnected from the parent plant by severe infection of both cortex and stele. These observations were later much amplified by J. Holden (1976), who found that hyphae travelling down the root advanced by growth through the stele, and that such hyphae grew faster than any others.

Our understanding of the infection habit of *G. graminis* has been increased by Holden's (1976) comparative study of infection habits of some related ectotrophic fungi on roots of Gramineae. One of these, *Phialophora graminicola*, was first isolated in Cambridge by P. R. Scott (1970) from grass roots; its biology was further studied by C. Balis (1970) and it is now known to be widespread and abundant in English grasslands (J. W. Deacon 1973*b*). It is a harmless parasite not impairing growth of its grass and cereal hosts; it grows over roots both superficially and by runner hyphae running intercellularly through the cortex but, unlike those of *G. graminis*, its hyphae do not enter the stele. The key to its non-pathogenic behaviour was discovered by Holden (1975) when he found that cortical cells of wheat and barley roots die much more quickly than had formerly been supposed. This fungus thus typically invades senescent cortical cells as a weak parasite. Death of cortical cells progresses inwards from the outermost to the innermost (6th) layer. By determining both depth of cortical cell death and position of the innermost runner hyphae in sections of roots infected by *P. graminicola*, Deacon (1980) demonstrated that runner hyphae grew "ectotrophically" outside the outermost layer of still living cortical cells.

These observations on *P. graminicola* are of agricultural interest, because both Scott (1970) and Balis (1970) showed that previous colonization of roots by this fungus could delay further progress of infections by the take-all fungus, *G. graminis*. The mechanism of this protective effect was explained by Speakman and Lewis (1978), who showed that infection of the outer cortex by *P. graminicola* caused a precocious lignification of both the inner tangential walls of the endodermis and of xylem cell-walls. This created a barrier that *G. graminis*, arriving later, was unable to pass. As J. W. Deacon (1973*b*, Table 2) has shown by means of wheat-seedling assays of soil samples, high populations of *P. graminicola* develop not only in old grassland but even in 1-year leys of grass with clover or another legume; these populations are maintained under several years of consecutive cropping with wheat or barley. Such populations of *P. graminicola*

exert a valuable biological control of take-all, because prior infection of a root by *P. graminicola* halts further advance by *G. graminis* along that particular root. This conclusion is supported by the extensive observations of D. B. Slope *et al.* (1978) of these two fungi on the roots of samples taken from 116 crops of winter wheat at Rothamsted; they suggested that the interposition of a grass and legume ley in the crop rotation would delay the onset of damaging take-all in a sequence of wheat crops. The use of 1-year leys in a cereal rotation is favoured by farmers for more general reasons, to which ecological control of take-all through increased populations of *P. graminicola* can now be added.

The ectotrophic infection habit is also displayed by many fungi causing serious diseases of bushes (e.g. the tea bush) and trees. The resistance, both passive and active, of their roots to infection is naturally much greater than that of roots of cereals and other annuals; this higher resistance is matched by a higher inoculum potential of the fungus, through mycelial aggregation into sheets (*Fomes annosus*), rhizomorphs (*Armillaria mellea*) or mycelial strands. As infection of a tree by *A. mellea* proceeds, host resistance declines in the later stage of disease and rhizomorphs may be found travelling in the plane of the cambium, rather than ectotrophically. The resistance of pine trees to *Fomes annosus* appears to be due to a strong production of resin in response to wounding or infection. But in some other species of conifer, such as Douglas fir (*Pseudotsuga taxifolia*), the resin-response to infection is much weaker, and ectotrophic infection is not needed for the overcoming of host resistance; in such conifers *F. annosus* is able to travel along a root through the inner wood cylinder, as J. Rishbeth (1951) was the first to demonstrate and his research associate, J. N. Gibbs (1968), was the first to explain by his studies of the resin response in various species of conifer. A full account of these ectotrophic root-infecting fungi can be found in Chapter 4 of my *Pathogenic Root-infecting Fungi* (Garrett, 1970).

MYCORRHIZAL FUNGI

In a memorable discussion entitled "Obligate parasitism in fungi", P.W. Brian (1967) distinguished between *ecologically obligate parasites* and *physiologically obligate parasites*. As an example of the first class, he cited *Gaeumannomyces graminis* var. *tritici*. This fungus can be isolated on

nutrient agar and maintained thereupon in axenic culture. But in the soil, it behaves as an ecologically obligate parasite; because of its low competitive saprophytic ability, it competes poorly with other fungi for colonization of dead plant tissues. Physiologically obligate parasites are those that cannot be maintained in axenic culture on dead culture media, though they can be grown on living tissues of a host plant by the process of tissue culture. This dependence on living tissues may be more than a purely nutritional one, which is why Brian defined such fungi as *physiologically* obligate. As he pointed out, the definition is a negative one, which for a particular fungal species may come to be inapplicable if the fungus is ultimately brought into culture as a saprophyte. This happened when Williams, Scott and Kuhl (1966) were the first to do this for *Puccinia graminis* f.sp. *tritici*, causing stem rust of wheat; growth in axenic culture was very slow, a fact that helps to explain failure of many previous attempts. Many members of the Uredinales (Basidiomycotina) still remain uncultured, nor have axenic cultures yet been obtained of any species in the Erysiphaceae (powdery mildews) of the Ascomycotina. Success in getting a fungus of this type into axenic culture and keeping it growing there represents a considerable technical achievement; it must not, however, mask the fact that all such fungi so far cultured grow very slowly by comparison with their speed of development in living host tissues. Such fungi still remain a class easily distinguished from ecologically obligate parasites like *G. graminis*, which grow well on nutrient agar.

This distinction has been well made by D. H. Lewis (1973) in a discussion entitled "Concepts in fungal nutrition and the origin of biotrophy". He has urged the general adoption of two useful categories of parasitism. *Necrotrophic parasites* are those that cause obvious physical damage to host tissues, and their ultimate destruction. Many specialized parasites belong to this class, which includes *G. graminis*, *Fomes annosus* and *Armillaria mellea*, discussed above. *Biotrophic parasites*, on the other hand, cause no physical damage to host tissues, though they do eventually cause *economic damage* to the host plant, because host nutrients are diverted to mycelial growth and eventual sporulation of the parasite. They may, however, cause distortion of host growth, by inducing (through production of growth hormones) the formation of galls and other types of abnormal growth. Thus the biotrophic fungus *Plasmodiophora brassicae* causes the clubroot disease of crucifers.

As a class, the mycorrhizal fungi exist in *mutualistic symbiosis* (for definition, see Lewis, 1973) with the roots of their host plants; all of them are biotrophic parasites in this symbiosis. A lucid general account of these fungi and the different types of mycorrhiza they form with host roots has been provided by Sarah E. Smith (1974), and is recommended as an introduction to this subject. A full treatment can be found in the second edition (1969) of J. L. Harley's *The Biology of Mycorrhiza*. The four main types of mycorrhiza recognized by D. H. Lewis (1973) and followed by S. E. Smith (1974) will now be described.

Sheathing mycorrhizas

These were formerly known as ectotrophic mycorrhizas, later shortened to ectomycorrhizas. The fungal partners are mostly basidiomycetes, with a small minority of ascomycetes. Such sheathing mycorrhizas occur chiefly on trees belonging to families Betulaceae, Fagaceae, Pinaceae and Myrtaceae, the last family including the large Australian genus of *Eucalyptus*. Mycorrhizal associations of this kind are formed by the nutrient-absorbing rootlets; by comparison with uninfected rootlets, such mycorrhizas are visibly swollen and are often forked and even coralloid through repeated dichotomous branching. In such a mycorrhiza, the cortex of the rootlet is surrounded by a sheath of fungal parenchyma, from which intercellular hyphae, constituting the "Hartig net" pass inwards through the cortex. Intracellular hyphae are rare in young mycorrhizas and the fungus does not invade the vascular tissues. These features can be seen in the transverse section shown in Fig. 17.

Fungi forming such sheathing mycorrhizas are biotrophic in their host-parasite relationship and ecologically obligate parasites in their mode of carbon (energy) nutrition. The renowned Swedish mycologist, Elias Melin (1948), discovered that most of these fungi that he studied were unable to degrade cellulose or lignin and required simple sugars as a carbon nutrient. D. H. Lewis and J. L. Harley (1965) showed that the fungal sheath of beech mycorrhizas converts host sucrose into trehalose and mannitol. Host cells cannot utilize either of these for their metabolism, which ensures that carbon nutrients can pass from host to fungus but not vice versa.

The reciprocal advantage obtained by the beech tree in this mutualistic symbiosis derives from the observed fact that fungal sheaths can absorb

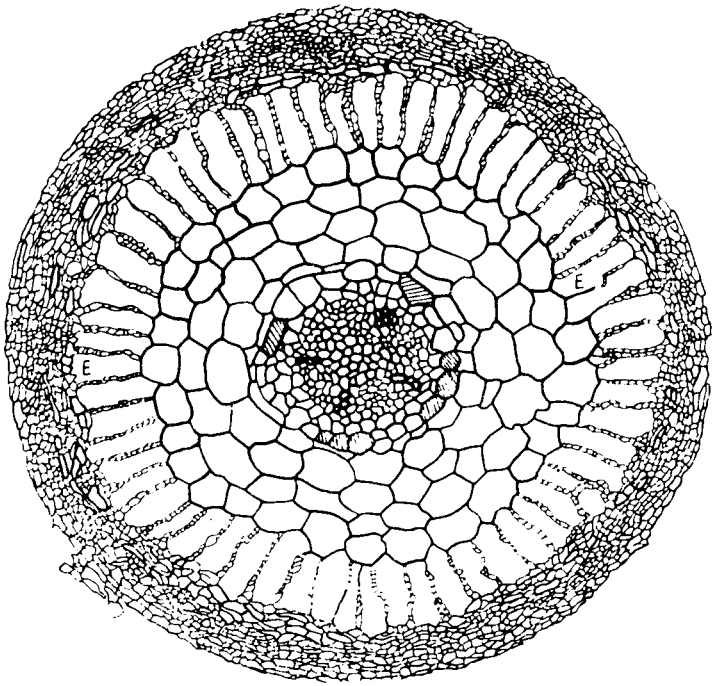

FIG. 17 Transverse section of mycorrhizal rootlet of *Fagus sylvatica*. Note "Hartig net" of fungal hyphae between radially elongated cells (E) of root epidermis (After F. A. L. Clowes (1951). By permission, *New Phytologist*).

mineral nutrients from infertile soils more efficiently than uninfected rootlets can do. This is particularly evident in phosphate-deficient soils; if such soils lack suitable fungi for the synthesis of mycorrhizas, as when trees are planted in soils formerly under arable farming for many years, then inoculation of the soil or young trees with such a fungus will increase tree growth, often dramatically. As explained in Chapter 2, much of the phosphate in soil is firmly bound to the soil complex of mineral and organic matter, so that plant roots find only a small proportion of total phosphate available in the soil solution for immediate uptake. In arable farming, this deficiency is remedied by periodical applications of a soluble phosphate fertilizer, usually some form of superphosphate. In forestry practice,

phosphate deficiency can be avoided by ensuring that suitable mycorrhizal fungi are present when young trees are planted.

Vesicular-arbuscular mycorrhizas

This group has been reviewed by Barbara Mosse (1973), one of the pioneers in this field of research. Mycorrhizal infection by these VA fungi is widespread among a majority of flowering-plant families. VA fungi are typical biotrophic parasites, which no one has yet succeeded in maintaining in axenic culture. Within the host cells they form *vesicles*, which are swollen, oil-filled cells terminating hyphae and functioning as a store of energy nutrients. The vesicles are produced later than are the *arbuscules*, which are formed after first invasion of a host cell and are generally digested by the cell later on. These arbuscules can be regarded as *haustoria*, which are special hyphae sent into host cells, from intercellular hyphae, by biotrophic parasites. Such haustoria enter the host cell but not its protoplast; the cytoplasmic membrane (Ch. 3) is invaginated by the haustorium. As some haustoria, including these arbuscules, are profusely branched, it was at first difficult to believe the evidence originally produced by Joan Fraymouth (1956) that haustoria of the Peronosporaceae invaginate but do not penetrate the cytoplasmic membrane of the host protoplast. She was able to demonstrate this by an ingenious combination of staining and plasmolysis techniques, whereby the stained protoplasts of the host cells were caused to contract away from the haustoria that had invaginated their bounding membranes. A drawing of haustoria is reproduced from her paper (Fig. 18).

Fraymouth's conclusion from her observations under the light microscope was later confirmed by electron-microscope studies of cell infection by other biotrophic fungal parasites. The advantage of this arrangement for biotrophic parasites is obvious; nutrient and other types of exchange between host and parasite can take place across their respective boundary membranes, but the parasite does not actually enter the host protoplast and so does not cause the gross physical damage that such an entry entails.

The fungi forming VA mycorrhizas belong to the Endogonaceae (Mastigomycotina) and some to the genus *Endogone*; the young fungal mycelium is thus aseptate. Mycorrhizal roots are usually surrounded by a mantle of hyphae extending outwards into the soil. The fungal mantle

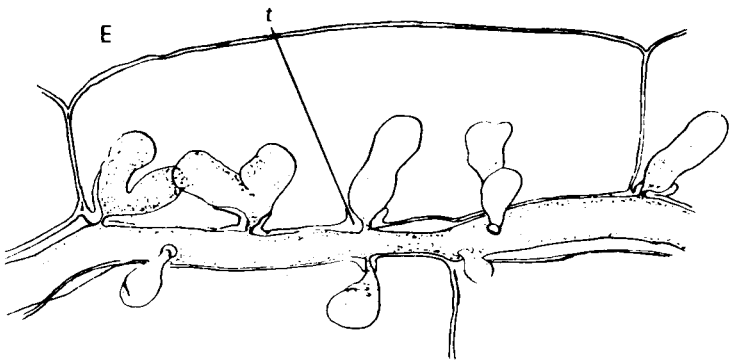

FIG. 18. Haustoria of *Peronospora parasitica* in cell of *Capsella bursa-pastoris*. *t* shows collar of thickening material deposited by host cell round neck of haustorium (after Joan Fraymouth (1956). By permission, *Transactions of the British Mycological Society*).

appears to be more efficient in absorbing mineral nutrients from infertile soils than are the root hairs of uninfected roots; this is especially evident in phosphate-deficient soils. Many experiments have now shown that, on such soils, this type of mycorrhizal infection results in better growth of infected, as compared with uninfected, plants. So, as with the sheathing mycorrhizas, the symbiosis is a mutualistic one; the fungus receives its supply of carbon nutrients and the host benefits by an improved supply of mineral nutrients on infertile soils.

Ericaceous mycorrhizas

There are two main types of these mycorrhizas, which according to host plants are styled ericoid and arbutoid, respectively (S. E. Smith, 1974). Much controversy once surrounded the fungal partners of the ericoid type, as seen on *Calluna vulgaris*; the fungus was claimed to be a species of *Phoma*, an imperfect genus in the Sphaeropsidales. The agar-plate method for isolating primary parasites from host tissue is notoriously unreliable, because the competitive saprophytic ability of specialized parasites for colonization of nutrient agar is often very poor (Ch. 7). But Pearson and Read (1973) evaded this difficulty by washing off propagules of other fungi in 20–25 changes of sterile water by the method of Harley and Waid (1955)

as described in Chapter 6. The macerated cortices of the washed roots were plated on agar, and *ca.* 95% of the fungal colonies were of slow-growing dark mycelia. The fungus was later identified as a cup-forming discomycete (Ascomycotina) and named *Pezizella ericacea.* Proof of the mycorrhizal relationship was provided by synthesizing it in axenic, two-membered culture of *P. ericacea* with *Calluna vulgaris.* The fungal symbiont is kept under control by periodic digestion of its mycelium in the cortical cells of the root. This slow-growing fungus is clearly an ecologically obligate parasite, deriving its carbon nutrients from the host. The host plant benefits on infertile podsols (Ch. 3) by increased efficiency of mycorrhizal *v* uninfected roots in uptake of both nitrogen and phosphate, as shown by Read and Stribley (1973).

Orchidaceous mycorrhizas

Orchid seedlings growing amongst natural vegetation are absolutely dependent for their development on infection by a suitable mycorrhizal fungus, usually a species of *Rhizoctonia. R. solani,* better known as a seedling pathogen, can serve as a fungal partner for *Dactylorchis purpurella* and several other British orchids. This dependence upon the fungal partner until the seedling becomes fully photosynthetic is due to the fact that orchid seeds are minute, each weighing only a few micrograms. In his book *The Reproductive Capacity of Plants,* E. J. Salisbury (1942) produced a large volume of data on individual seed weight and average seed-producing capacity of a single plant for many species of flowering plant. From all this, he concluded that mean seed-size for any species was closely related to the nutrient reserve needed by the seedling until it has become photosynthetically self-supporting. Thus seedlings colonizing open habitats of normal light intensity tend to produce large numbers of small seeds. But seedlings of species growing in closed habitats, as under a woodland canopy, tend to produce smaller numbers of larger seeds, because the seedling has to be maintained by the endogenous reserves of the seed for a longer period before photosynthesis can completely support further growth. As Salisbury pointed out, maximum efficiency in deployment of reproductive resources demands that average seed-size in any species should suffice for the seedling to grow up to a size in which photosynthesis can support it in the typical habitat of the species; a seed-size larger than this is wasteful of

reproductive capacity. The smaller the seed size, then the larger the number of seeds that can be produced. A large seed-output is a characteristic that leads to greater abundance and wider distribution of a plant species, and so has a substantial survival-value for the species as a whole. As part of his general argument, Salisbury pointed to the Orchidaceae, in which an external mode of seedling nutrition, through mycotrophy, has enabled a dramatic reduction in seed size to be evolved; in some orchid species, four million seeds may be produced in one seed capsule alone.

Amongst orchid seeds germinating in a suitable habitat, only a proportion will make contact with a compatible species of *Rhizoctonia*. Amongst these, only a small proportion will establish a balanced relationship with the fungus. In some situations, the inoculum potential of the fungus is too high and it parasitizes and kills the seedling. In other encounters, the defence reaction of the seedling is too strong and it rejects the attempted infection. But in a small minority of seedlings, a balanced relationship develops in the young protocorm and the roots arising from it. The fungus is confined to parenchyma cells of its host, in which it forms coils of hyphae (pelotons); it behaves as a biotroph and the hyphae are enclosed by the invaginated cytoplasmic membrane of the host cell. Infection is kept under control by host-cell reactions, and the fungal pelotons are eventually digested.

In orchid mycorrhizas, we find a strange reversal of the usual nutritional exchange between fungal parasite and seedling host, as exemplified by infections by *Rhizoctonia solani* of seedlings other than those of orchids. The minute orchid seedling has little to give the fungus; instead, it takes all from it. For some time, therefore, the orchid seedling is completely dependent on its fungal partner; in achlorophyllous orchids, formerly styled "saprophytic", the orchid is a parasite upon the fungus throughout its life. By tradition, which I have no wish unnecessarily to discard, the orchid – fungus relationship is called a "mycorrhizal" one, but it is not a mutualistic symbiosis; the orchid is a parasite of the fungus, which is living either as a saprophyte or, sometimes, as a parasite of another flowering plant.

A laboratory model of the orchid – fungus relationship has been demonstrated by S. E. Smith (1966) who showed that both *Rhizoctonia solani* and *R. repens* were able to colonize filter-paper cellulose buried in soil, in competition with the rest of the soil microflora. Secondly she

showed that *R. repens*, growing in axenic culture on cellulose, was able to promote a continued vigorous development of the seedlings of *Dactylorchis purpurella* and *D. praetermissa*.

Such studies as these on the widespread mycorrhizal relationship show why it is that soil mycologists rarely abandon their chosen field of research. To those who may contemplate embarking on such studies, I can promise an infinite variety of problems and also the prospect of contributing something towards the maintenance of soil fertility, on which all our lives ultimately depend and for which I have written this book.

BIBLIOGRAPHY

ADAMETZ, I. (1886) *Untersuchungen über die niederen Pilze der Ackerkrume*, Inaug. Diss., Leipzig.

AINSWORTH, G. C. (1971) *Ainsworth and Bisby's Dictionary of the Fungi*, 6th ed., Commonwealth Mycological Institute, Kew.

BAKER, R. (1978) In *Plant Disease*, J. G. Horsfall & E. B. Cowling (Eds.), Vol. 2, pp. 137–57, Academic Press, New York.

BALIS, C. (1970) *Ann. appl. Biol.* **66**, 59–73.

BARNETT, H. L. and HUNTER, B. B. (1972) *Illustrated Genera of Imperfect Fungi*, 3rd ed., Burgess, Minneapolis.

BATEMAN, D. F. (1964) *Phytopathology* **54**, 1372–7.

BEVERIDGE, W. I. B. (1950) *The Art of Scientific Investigation*, Heinemann, London.

BISHOP, O. N. (1966) *Statistics for Biology*, Longmans, London.

BLAKESLEE, A. F. (1904) *Proc. Am. Acad. Arts Sci.* **40**, 205–319.

BRASIER, C. M. and SANSOME, E. (1975) *Trans. Br. mycol. Soc.* **65**, 49–65.

BRIAN, P. W. (1949) *Ann. Bot.* **13**, 59–77.

BRIAN, P. W. (1967) *Proc. R. Soc. B* **168**, 101–18.

BROWN, M. E. (1974) *A. Rev. Phytopath.* **12**, 181–97.

BURGES, A. (1939) *Broteria* **8**, 64–81.

BURGES, A. and NICHOLAS, D. P. (1961) *Soil Sci.* **92**, 25–9.

BURNETT, J. H. (1976) *Fundamentals of Mycology*, 2nd ed., Arnold, London.

BUTLER, E. J. (1907) *Mem. Dep. Agric. India, Bot. Ser. I*, **5**.

BUTLER, F. C. (1953*a*) *Ann. appl. Biol.* **40**, 284–304.

BUTLER, F. C. (1953*b*) *Ann. appl. Biol.* **40**, 305–11.

BUTLER, F. C. (1959) *Ann. appl. Biol.* **47**, 28–36.

BUTLER, G. M. (1957) *Ann. Bot.* **21**, 523–37.

BUTLER, G. M. (1958) *Ann. Bot.* **22**, 219–36.

BUTLER, G. M. (1961) *Ann. Bot.* **25**, 341–52.

CARLILE, M. J. (1980) In *The Eukaryotic Microbial Cell*, Symposium 30, Soc. Gen. Microbiol., G. W. Gooday, D. Lloyd & A. P. J. Trinci (Eds.), pp. 1–40, Cambridge.

CATEN, C. E. and DAY, A. W. (1977) *A. Rev. Phytopath.* **15**, 295–318.

CHANG, YUNG and HUDSON, H. J. (1967) *Trans. Br. mycol. Soc.* **50**, 649–66.

CHESTERS, C. G. C. (1940) *Trans. Br. mycol. Soc.* **24**, 352–5.

CHESTERS, C. G. C. (1948) *Trans. Br. mycol. Soc.* **30**, 100–17.

CHESTERS, C. G. C. and THORNTON, R. H. (1956) *Trans. Br. mycol. Soc.* **39**, 301–13.

CHOLODNY, N. (1930) *Arch. Microbiol.* **1**, 620–52.

CONN, H. J. (1917) *Science, N.Y.* **44**, 857–8.

CONN, H. J. (1922) *Soil Sci.* **14**, 149–51.

CONN, H. J. (1932) *Tech. Bull. N.Y. St. agric. Exp. Sta.* **204**.
COOK, R. J. and PAPENDICK, R. I. (1970) In *Root Diseases and Soil-borne Pathogens*, T. A. Toussoun, R. V. Bega & P. E. Nelson (Eds.), pp. 81–8, Univ. of California Press, Berkeley.
CORNER, E. J. H. (1932*a*) *Ann. Bot.* **46**, 71–111.
CORNER, E. J. H. (1932*b*) *Trans. Br. mycol. Soc.* **17**, 51–81.
CORNER, E. J. H. (1950) *A Monograph of Clavaria and Allied Genera*, Oxford.
CUNNINGHAM, G. H. (1947) *N.Z. J. Sci. Tech. A* **28**, 238–51.
CUNNINGHAM, G. H. (1954) *Trans. Br. mycol. Soc.* **37**, 44–50.
DARWIN, CHARLES (1881) *The Formation of Vegetable Mould through the Action of Worms*, London.
DEACON, J. W. (1973*a*) *Soil Biol. Biochem.* **5**, 339–53.
DEACON, J. W. (1973*b*) *Trans. Br. mycol. Soc.* **61**, 471–85.
DEACON, J. W. (1976) *Trans. Br. mycol. Soc.* **66**, 383–91.
DEACON, J. W. (1979) *Trans. Br. mycol. Soc.* **72**, 469–77.
DEACON, J. W. (1980) *Trans. Br. mycol. Soc.* **74**,
EGGINS, H. O. W. and PUGH, G. J. F. (1962) *Nature, Lond.* **193**, 94–5.
FALCK, R. (1912) *Hausschwammforsch.* **6**, 1–405.
FERRAZ, J. F. P. (1973) *Trans. Br. mycol. Soc.* **61**, 237–49.
FISHER, R. A. (1930) *The Genetical Theory of Natural Selection*, Oxford.
FORBES, R. S. and DICKINSON, C. H. (1977) *Trans. Br. mycol. Soc.* **68**, 229–35.
FRAYMOUTH, J. (1956) *Trans. Br. mycol. Soc.* **39**, 79–107.
GARRETT, S. D. (1934) *J. Agric. S. Aust.* **37**, 664–74.
GARRETT, S. D. (1936) *Ann. appl. Biol.* **23**, 667–99.
GARRETT, S. D. (1937) *Ann. appl. Biol.* **24**, 747–51.
GARRETT, S. D. (1938) *Ann. appl. Biol.* **25**, 742–66.
GARRETT, S. D. (1940) *Ann. appl. Biol.* **27**, 199–204.
GARRETT, S. D. (1944) *Ann. appl. Biol.* **31**, 186–91.
GARRETT, S. D. (1950) *Biol. Rev.* **25**, 220–54.
GARRETT, S. D. (1952) *Sci. Progr.* **40**, 436–50.
GARRETT, S. D. (1953) *Ann. Bot.* **17**, 63–79.
GARRETT, S. D. (1956) *Biology of Root-infecting Fungi*, Cambridge.
GARRETT, S. D. (1962) *Trans. Br. mycol. Soc.* **45**, 115–20.
GARRETT, S. D. (1966) *Trans. Br. mycol. Soc.* **49**, 57–68.
GARRETT, S. D. (1967) *Trans. Br. mycol. Soc.* **50**, 519–24.
GARRETT, S. D. (1970) *Pathogenic Root-infecting Fungi*, Cambridge.
GARRETT, S. D. (1971) *Trans. Br. mycol. Soc.* **57**, 121–8.
GARRETT, S. D. (1975) *Soil Biol. Biochem.* **7**, 323–7.
GARRETT, S. D. (1976) *Soil Biol. Biochem.* **8**, 229–34.
GARRETT, S. D. (1978) *Trans. Br. mycol. Soc.* **70**, 21–7.
GARRETT, S. D. (1980) *Trans. Br. mycol. Soc.* **74**, 259–63.
GARRETT, S. D. (1981) In *The Biology and Control of Take-all*, M. J. C. Asher & P. J. Shipton (Eds.), Academic Press, London.
GIBBS, J. N. (1968) *Ann. Bot.* **32**, 649–65.
GREENWOOD, D. J. (1970) *J. Soil Sci.* **21**, 314–29.
GREGORY, P. H. (1973) *The Microbiology of the Atmosphere*, 2nd ed., Leonard Hill, Aylesbury.
GRIFFIN, D. M. (1963) *Biol. Rev.* **38**, 141–66.
GRIFFIN, D. M. (1968) *Trans. Br. mycol. Soc.* **51**, 319–22.

GRIFFIN, D. M. (1972) *Ecology of Soil Fungi*, Chapman & Hall, London.
GRIFFITHS, E. (1965) *Biol. Rev.* **40**, 129–42.
HANSEN, H. N. (1938) *Mycologia* **30**, 442–55.
HANSEN, H. N. and SMITH, R. E. (1932) *Phytopathology* **22**, 953–64.
HARLEY, J. L. (1969) *The Biology of Mycorrhiza*, 2nd ed., Leonard Hill, London.
HARLEY, J. L. (1973) *J. Nat. Sci. Coun. Sri Lanka* **1**, 31–48.
HARLEY, J. L. (1978) *Proc. R. Soc. B* **203**, 1–21.
HARLEY, J. L. and WAID, J. S. (1955) *Trans. Br. mycol. Soc.* **38**, 104–18.
HEDGER, J. N. and HUDSON, H. J. (1974) *Trans. Br. mycol. Soc.* **62**, 129–43.
HENIS, Y., KELLER, P. and KEYNAN, A. (1961) *Can. J. Microbiol.* **7**, 857–63.
HILTNER, L. (1904) *Arb. dtsch. Landw-Ges.* **98**, 59–78.
HOLDEN, J. (1975) *Soil Biol. Biochem.* **7**, 333–4.
HOLDEN, J. (1976) *Soil Biol. Biochem.* **8**, 109–19.
HORNBY, D. (1969) *J. appl. Bact.* **32**, 244–58.
HUDSON, H. J. (1968) *New Phytol.* **67**, 837–74.
HUDSON, H. J. (1980) *Fungal Saprophytism*, 2nd ed., Arnold, London.
IKEDIUGWU, F. E. O., DENNIS, C. and WEBSTER, J. (1970) *Trans. Br. mycol. Soc.* **54**, 307–9.
IKEDIUGWU, F. E. O. and WEBSTER, J. (1970) *Trans. Br. mycol. Soc.* **54**, 181–210.
INGOLD, C. T. (1971) *Fungal Spores*, Oxford.
IRVINE, W. (1955) *Apes, Angels and Victorians*, Weidenfeld & Nicolson, London.
JACKS, G. V. (1954) *Soil*, Nelson, London.
JENNINGS, D. H. (1981) In *Decomposition by Basidiomycetes*, J. C. Frankland & J. N. Hedger (Eds.). Symposium 4, Br. mycol. Soc., Cambridge.
JENSEN, H. L. (1934–36) *Proc. Linn. Soc. N.S.W.* **59**, 101, 200; **60**, 145; **61**, 27.
JONES, P. C. T. and MOLLISON, J. E. (1948) *J. gen. Microbiol.* **2**, 54–69.
KEEN, B. A. and RACZKOWSKI, H. (1921) *J. agric. Sci.* **11**, 441–9.
KNIEP, H. (1920) *Verh. phys.-med. Ges. Würzburg* **46**, 1–18.
KNIEP, H. (1922) *Verh. phys.-med. Ges. Würzburg* **47**, 1–28.
KUBIENA, W. L. (1938) *Micropedology*, Collegiate Press, Ames, Iowa.
LEWIS, D. H. (1973) *Biol. Rev.* **48**, 261–78.
LEWIS, D. H. and HARLEY, J. L. (1965) *New Phytol.* **64**, 224–69.
LUCAS, R. L. (1955) *Ann. appl. Biol.* **43**, 134–43.
LUCAS, R. L. (1977) *Trans. Br. mycol. Soc.* **69**, 1–9.
MACER, R. C. F. (1961*a*) *Ann. appl. Biol.* **49**, 152–64.
MACER, R. C. F. (1961*b*) *Ann. appl. Biol.* **49**, 165–72.
MACNISH, G. C. (1973) *Aust. J. biol. Sci.* **26**, 1319–25.
MASON, E. W. (1937) *Annotated Account of Fungi received at the Imperial Mycological Institute*, List II, Fasc. 3–General Part, Commonwealth Mycological Institute, Kew.
MELIN, E. (1948) *Trans. Br. mycol. Soc.* **30**, 92–9.
MOSSE, B. (1973) *A. Rev. Phytopath.* **11**, 171–96.
MOTTA, J. J. (1969) *Am. J. Bot.* **56**, 610–9.
MOTTA, J. J. (1971) *Am. J. Bot.* **58**, 80–7.
MUELLER, K. E. and DURRELL, L. W. (1957) *Phytopathology* **47**, 243.
OLD, K. M. (1977) *Trans. Br. mycol. Soc.* **68**, 277–81.
OUDEMANS, C. A. J. A. and KONING, C. J. (1902) *Arch. Néerl. Sci., Nat. Ser. 2*, **7**, 266–98.
PARKINSON, D., GRAY, T. R. G. and WILLIAMS, S.T. (1971) *Methods for Studying the Ecology of Soil Micro-organisms*, IBP Handb. **19**, Blackwell, Oxford.
PARKINSON, D. and KENDRICK, W. B. (1960) In *The Ecology of Soil Fungi*, D. Parkinson & J. S. Waid (Eds.), pp. 22–8, Liverpool Univ. Press.

PARKINSON, D. and WILLIAMS, S. T. (1961) *Pl. Soil* **13**, 347–55.
PEARSON, V. and READ, D. J. (1973) *New Phytol.* **72**, 371–9.
PIROZYNSKI, K. A. (1976) *A. Rev. Phytopath.* **14**, 237–46.
PODGER, F. D. (1975) In *Biology and Control of Soil-borne Plant Pathogens*, G. W. Bruehl (Ed.), pp. 27–36. American Phytopathological Society, St. Paul, Minnesota.
PONTECORVO, G. (1956) *A. Rev. Microbiol.* **10**, 393–400.
PONTECORVO, G., ROPER, J. A. and FORBES, E. (1953) *J. gen. Microbiol.* **8**, 198–210.
RAO, A. S. (1959) *Trans. Br. mycol. Soc.* **42**, 97–111.
READ, D. J. and STRIBLEY, D. P. (1973) *Nature, Lond.* **244**, 81.
REINKING, O. A. (1935) *Zbl. Bakt.* **91**, 243–55.
RISHBETH, J. (1951) *Forestry* **24**, 114–20.
RISHBETH, J. (1957) *Ann. Bot.* **21**, 215–45.
RISHBETH, J. (1963) *Ann. appl. Biol.* **52**, 63–77.
RISHBETH, J. (1968) *Trans. Br. mycol. Soc.* **51**, 575–86.
RISHBETH, J. and NAYLOR, A. G. (1957) *Ann. Bot.* **21**, 599–609.
ROSSI, G. (1928) *Ital. agric.* 1928, no. 4.
RUSSELL, E. J. and HUTCHINSON, H. B. (1909) *J. agric. Sci.* **3**, 111–44.
RUSSELL, E. W. (1973) *Soil Conditions and Plant Growth*, 10th ed., Longmans, London.
SADASIVAN, T. S. (1939) *Ann. appl. Biol.* **26**, 497–508.
SALISBURY, E. J. (1942) *The Reproductive Capacity of Plants*, Bell, London.
SANSOME, E. (1963) *Trans. Br. mycol. Soc.* **46**, 63–72.
SCHOFIELD, R. K. (1935) *Trans. 3rd int. Congr. Soil Sci.* **2**, 37–48.
SCHÜTTE, K. H. (1956) *New Phytol.* **55**, 164,–82.
SCOTT, P. R. (1970) *Trans. Br. mycol. Soc.* **55**, 163–7.
SEWELL, G. W. F. (1959) *Trans. Br. mycol. Soc.* **42**, 312–21.
SLOPE, D. B., SALT, G. A., BROOM, E. W. and GUTTERIDGE, R. J. (1978) *Ann. appl. Biol.* **88**, 239–46.
SMITH, A. M. (1976) *Soil Biol. Biochem.* **8**, 293–8.
SMITH, S. E. (1966) *New Phytol.* **65**, 488–99.
SMITH, S. E. (1974) *Crit. Rev. Microbiol.* **3**, 275–313.
SPEAKMAN, J. B. and LEWIS, B. G. (1978) *New Phytol.* **80**, 373–80.
STANIER, R. Y., ADELBERG, E. A. and INGRAHAM, J. L. (1977) *General Microbiology*, 4th ed., Macmillan, London.
STOTZKY, G. and MARTIN, R. T. (1963) *Pl. Soil* **18**, 317–37.
STOTZKY, G. and REM, L. T. (1966) *Can. J. Microbiol.* **12**, 547–63.
TEIXEIRA, A. R. (1962) *Biol. Rev.* **37**, 51–81.
THOM, C. and MORROW, M. B. (1937) *J. Bact.* **33**, 77–8.
THORNTON, R. H. (1952) *Research, Lond.* **5**, 190–1.
TRIBE, H. T. (1957) In *Microbial Ecology*, 7th Symp. Soc. Gen. Microbiol., pp. 287–98, Cambridge.
TRIBE, H. T. (1960) In *The Ecology of Soil Fungi*, D. Parkinson & J. S. Waid (Eds.), pp. 246–56, Liverpool Univ. Press.
TRIBE, H. T. (1966) *Trans. Br. mycol. Soc.* **49**, 457–66.
TRINCI, A. P. J. (1971) *J. gen. Microbiol.* **67**, 325–44.
TRUJILLO, E. E. (1963) *Phytopathology* **53**, 162–6.
WAKSMAN, S. A. (1929) *Principles of Soil Microbiology*, Baillière, Tindall & Cox, London.
WARCUP, J. H. (1950) *Nature, Lond.* **166**, 117.
WARCUP, J. H. (1951) *Trans. Br. mycol. Soc.* **34**, 376–99.
WARCUP, J. H. (1955*a*) *Trans. Br. mycol. Soc.* **38**, 298–301.

WARCUP, J. H. (1955*b*) *Nature, Lond.* **175**, 953.
WARCUP, J. H. (1957) *Trans. Br. mycol. Soc.* **40**, 237–59.
WARCUP, J. H. (1960) In *The Ecology of Soil Fungi*, D. Parkinson & J. S. Waid (Eds.), pp. 3–21, Liverpool Univ. Press.
WARDLAW, C. W. (1941) *Nature, Lond.* **147**, 380–1.
WASTIE, R. L. (1961) *Trans. Br. mycol. Soc.* **44**, 145–59.
WATKINSON, S. C. (1971*a*) *New Phytol.* **70**, 1079–88.
WATKINSON, S. C. (1971*b*) *Trans. Br. mycol. Soc.* **57**, 535–9.
WATKINSON, S. C. (1979) In *Fungal Walls and Hyphal Growth*, J. H. Burnett & A. P. J. Trinci (Eds.), pp. 93–113, Cambridge.
WEBSTER, J. (1970) *Trans. Br. mycol. Soc.* **54**, 161–80.
WHITEHOUSE, H. L. K. (1949*a*) *Biol. Rev.* **24**, 411–47.
WHITEHOUSE, H. L. K. (1949*b*) *New Phytol.* **48**, 212–44.
WHITEHOUSE, H. L. K. (1951) *Indian Phytopath.* **4**, 91–105.
WHITEHOUSE, H. L. K. (1965) *Towards an Understanding of the Mechanism of Heredity*, Arnold, London.
WILLIAMS, P. G., SCOTT, K. J. and KUHL, J. L. (1966) *Phytopathology* **56**, 1418–9.

INDEX